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THE REPRODUCTIVE BIOLOGY AND DEVELOPMENT OF TWELVE SPECIES OF HOLOTHUROIDS FROM THE SAN JUAN ISLANDS, WASHINGTON

by

ⓒ F. SCOTT MCEUEN

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

ZOOLOGY

EDMONTON, ALBERTA
SPRING 1986

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE REPRODUCTIVE BIOLOGY AND DEVELOPMENT OF TWELVE SPECIES OF HOLOTHUROIDS FROM THE SAN JUAN ISLANDS, WASHINGTON submitted by F. SCOTT MCEUEN in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

Supervisor

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Date 2 December 1985

ABSTRACT

A comparative study of the reproductive biology and development of 12 species of holothuroids in the San Juan Islands, Washington, was conducted at Friday Harbor Laboratories during the period May 1981 through August 1983. The major areas of research focus were spawning behavior, breeding season, egg size, fecundity, larval development, length of pelagic period, and size at metamorphosis. The results of the research were combined with information from the literature on holothuroid reproduction for a delineation of the reproductive and developmental patterns in the class.

Observations of reproductive behavior in the field and laboratory revealed a diversity of spawning behaviors including tentacle waving, swaying, raising the anterior half of the body off the substrate, and spewing gametes. Pronounced tentacle movements ensnaring the gametes, and swaying, both occurred during low current conditions when gametes fell on the buccal membrane. A literature review of holothuroid spawning revealed that species living in low current habitats forcefully expel their gametes. Males preceded females in gamete release and both sexes are involved in synchronized, mass field spawnings. Spontaneous laboratory spawnings of ripe *Psolus chitonoides*, *Cucumaria miniata*, and *Eupentacta quinquesemita* were clumped in the morning, afternoon, and late evening, respectively. The syncopated spawning among these three sympatric species is interpreted as adaptive behavior to minimize heterospermy. The reproductive behaviors exhibited by holothuroids are thought to increase fertilization rates by synchronizing gamete release, releasing sexual products when current regimes are favorable, timing spawning to avoid interspecific gamete mixing, and aiding gamete mixing through swaying, tentacle movements, pseudocopulation, and forcible discharge of gametes.

Breeding seasons were approximated from data on field and laboratory spawning, qualitative assessments of gonad ripeness, and previous reports of reproductive activity. The three brooding species (*Cucumaria lubrica*, *Cucumaria pseudocurata*, *Leptosynapta clarki*) bred in late fall and early winter (late October - early January), dendrochirotids with lecithotrophic larvae spawned in late winter through spring (mid Febuary - early June), and the single aspidochirotid (with planktotrophic auricularia) had an extended season from spring to mid-summer (April to early August). *Molpadia intermedia* had a breeding period of November through December.

After fertilization, the 267 um, telolecithal egg of Molpadia intermedia developed into a doliolaria with two ciliary rings. The pentactula settled at 95 - 96 hr and metamorphosed into a transparent juvenile with spired tri-radiate ossicles. Egg sizes and a similar larva in other molpadiids predict similar development for the entire order.

Eggs of Cucumaria miniata, C. piperata, Cucumaria sp., Eupentacta quinquesemita, Psolidium bullatum, and Psolus chitonoides were 330 - 627 um in diameter and produced a doliolaria with three ciliary rings except for the uniformly ciliated larva of E. quinquesemita. In their development, the gastrula was followed by an evenly ciliated early larva, which then formed ciliary rings and primary tentacles, and settled. The appearance of ciliary rings and tentacles just prior to settlement suggests they are employed in descent out of the water column, habitat selection, and attachment. The absence of phototactic larval behavior in E. quinquesemita is associated with uniform ciliation of the larva, settlement sites in the field, and with adult habitat (on top of hard substrata). Ringed doliolaria larvae of species living under cobble were photonegative and settled on the undersides of rocks. The positively phototactic doliolaria of P. populifera settled on top of the mud.

Holothuroid developmental patterns include a planktotrophic auricularia, lecithotrophic doliolaria, pelagic direct development, external brooding, coelomic brooding, and ovarian brooding. Lecithotrophic doliolariae and external brooding characterize dendrochirotids, auriculariae are typical of aspidochirotids, and combinations of feeding and non-feeding larvae and coelomic and ovarian brooding are found in the apodids. Species with auriculariae breed primarily in summer, those with doliolariae in spring (some in summer), external brooders in winter, coelomic brooders are continuous breeders, and most deep-sea forms also appear to breed continuously. Egg size is associated with developmental type, pelagic period, and size at metamorphosis. The original pattern of holothuroid development is thought to be a planktotrophic auricularia that metamorphoses into a doliolaria with ciliary rings and then into a benthic juvenile, from which all other patterns are derived.

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I. INTRODUCTION

The research reported in this thesis is a comparative study of the reproduction and development of 12 species of holothuroids from the San Juan Islands, Washington, including *Cucumaria lubrica* (Clark, 1901); *C. miniata* (Brandt, 1835); *C. piperata* (Stimpson, 1864); *C. pseudocurata* Deichmann, 1938; *Cucumaria* sp. (unidentified); *Eupentacta quinquesemita* (Selenka, 1867); *Pentamera populifera* Ohshima, 1915; *Psolidium bullatum* Ohshima, 1915; *Psolus chitonoides* Clark, 1901; *Parastichopus californicus* (Stimpson, 1857); *Leptosynapta clarki* Heding, 1928, and; *Molpadia intermedia* (Ludwig, 1894). There are numerous justifications for this type of study, one of them being that sea cucumbers comprise a very widespread and abundant class of echinoderms. Their morphological, behavioral, and habitat diversity are unequalled among the Echinodermata. Body sizes range from meiofaunal synaptids 2 mm in length (Swedmark, 1971) to tropical synaptids 5 m in length (Mortensen, 1938). Other extremes are the gelatinous, pelagic, deep-sea elasipodids (Hansen, 1975; Pawson, 1976) and the sessile psolids covered with heavy calcareous plates (Pawson, 1967). Aside from their more common habitats of mud, sand, shell, rock walls, boulders, cobble fields, algal fronds and holdfasts, coral reefs, and shallow lagoons, holothuroids are also found living on live cockles (Dakin, 1960), sponges (Cherbonnier and Feral, 1984), mangrove roots (Clark, 1896a,b), docks and pilings (chapter five), mussel beds (Ohshima, 1916), eel grass (Deichmann, 1930), fish (as a commensal; Martin, 1969a,b), in the interstices of sand and mud (Swedmark, 1971), and in the water column (Barnes et al., 1976). In their extensive treatment of deep-sea life, Heezen and Hollister (1971) stated that "sea cucumbers are the largest and most numerous animals seen on the deep-sea floor" and came to the "inescapable conclusion that holothurians are the dominant large animals of the major part of the earth's surface."

In addition to their ubiquity, holothuroids are economically important as a foodstuff. They form the basis of large fisheries in the Indo-west Pacific, China, and Japan where they are prepared using special processes of drying and smoking and then marketed as trepang or beche-de-mer.

A third reason supporting the value of this study is the ecological role played by holothuroids. In the northwest Pacific, which supports one of the world's richest echinoderm faunas, sea cucumbers (mostly dendrochirotids) frequently dominate benthic communities in numbers and biomass in mud, sand, and rocky substrata. One such dendrochirotid, Cucumaria lubrica, attains great population densities and is considered a "key-industry" species for a predator-prey system (Birkeland et al., 1982).

The primary motive, however, for undertaking this research project is to increase our knowledge of the reproduction and development of the Holothuroidea. In the past, these two subjects, or areas within them, have been reviewed in varying degrees of thoroughness by a number of authors (Bronn, 1889-1892; Bury, 1889; Bather et al., 1900; Clark, 1902; Ludwig, 1904; MacBride, 1914; Ohshima, 1921; Vaney, 1925; Cuenot, 1948; Hyman, 1955; Boolootian, 1966; Fell, 1967; Inaba, 1968). All of these reviews have been hampered by the lack of information, stemming largely from the fact that the majority of studies on sea cucumber reproduction are brief and incomplete. A more thorough, up-to-date summary is also needed because even the most comprehensive treatment by Hyman (1955) has not included much useful information; in addition, the intervening 30 years since her major treatise have yielded additional material that requires incorporation into the earlier literature.

The value of comparative studies is illustrated in the works of Theodore Mortensen (1921, 1931, 1937, 1938) and Gunnar Thorson (1946) whose comprehensive approaches to echinoderm and general marine invertebrate reproduction stand as classics and are still frequently cited in current literature. I feel, therefore, that a broad comparative approach examining a number of species will make a fundamental as well as substantial contribution to a major group of invertebrates whose reproductive biology is poorly known. The array of species I have chosen for study are all abundant in the waters of the San Juan Archipelago; relatively little has been recorded on their reproductive habits and early life histories. For four species (Cucumaria sp., Pentamera populifera, Psolidium bullatum, and Molpadia intermedia), nothing is known of their reproduction. Only fragmentary information exists (Mortensen, 1921; Courtney, 1927; Johnson, 1931; Johnson, 1932; Johnson and Johnson, 1950; Fernald, 1959; Strathmann, 1978) for four other species (C. miniata, C. piperata, Eupentacta quinquesemita and Parastichopus californicus). Unpublished theses (Jones, 1960; Everingham, 1961) treat early embryological stages of Psolus chitonoides and some aspects of ovarian brooding in Leptosynapta clarki, respectively. Studies of C. lubrica (Engstrom, 1982) and C.

pseudocurata (Smith, 1962; Rutherford, 1973) have examined selected details of spawning and brooding behavior.

This thesis is organized into five chapters. The opening chapter (two) describes the different spawning behaviors and outlines breeding seasons. A substantial discussion deals with the significance of holothuroid reproductive behaviors and their periodicity, spawning mechanisms, gamete properties, secondary sexual characteristics, and the proximate and ultimate factors governing reproduction. The third chapter describes the development of Molpadia intermedia and reviews reproduction in the molpadiids. The development of two psolids, Psolus chitonoides and Psolidium bullatum, is detailed in the fourth chapter with an examination of reproductive patterns in the family Psolidae. The fifth chapter compares the larval development of seven dendrochirotids, makes an overview of modes of development among the Dendrochirotida, and uses comparative data as a basis for a hypothesis on the significance of ciliation patterns on dendrochirotid larvae. The final chapter is a comprehensive review of reproduction and development in the Holothuroidea. Each order is taken separately and patterns evident from the available information are outlined and discussed. Characteristics such as breeding season, egg size, fecundity, mode of development, length of development, larval pelagic period, size at metamorphosis, and adult distribution are included. In each chapter, a thorough examination of all relevant literature on holothuroids was conducted, as far as was possible.

Employing the comparative approach has an advantage in that it frequently reveals patterns which may not otherwise be readily apparent. It also stimulates new ideas and avenues of research; numerous projects suggest themselves throughout this thesis. It is my hope that the work presented here will contribute to our knowledge of and stimulate more study in the biology of holothuroids and echinoderms in general.

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II. SPAWNING BEHAVIORS AND BREEDING SEASONS OF 12 SPECIES OF HOLOTHUROIDS (ECHINODERMATA) FROM THE SAN JUAN ARCHIPELAGO, WASHINGTON

A. INTRODUCTION

Various ultimate and proximate factors have been proposed as agents controlling reproductive events in marine invertebrates, but there has been little success in isolating the specific environmental cues (zeitgebers) that entrain gametogenic cycles and trigger spawning episodes in echinoderms (Giese and Pearse, 1974; Himmelman, 1975; Lessios, 1981; Pearse and Eernisse, 1982). Investigations into the natural factors that induce spawning in echinoderms have been necessarily limited due to the difficulty of securing in situ behavioral data, the seasonal reproduction exhibited by most echinoderms (see reviews by Hyman, 1955; Boolootian, 1966; Reese, 1966), and the technical obstacles encountered in experimentally separating the environmental variables potentially responsible for initiating spawning activities. Consequently, most observations for spontaneous spawnings of echinoderms are anecdotal and based on laboratory phenomena, lacking descriptive detail and recognition of the significance of the reproductive behaviors.

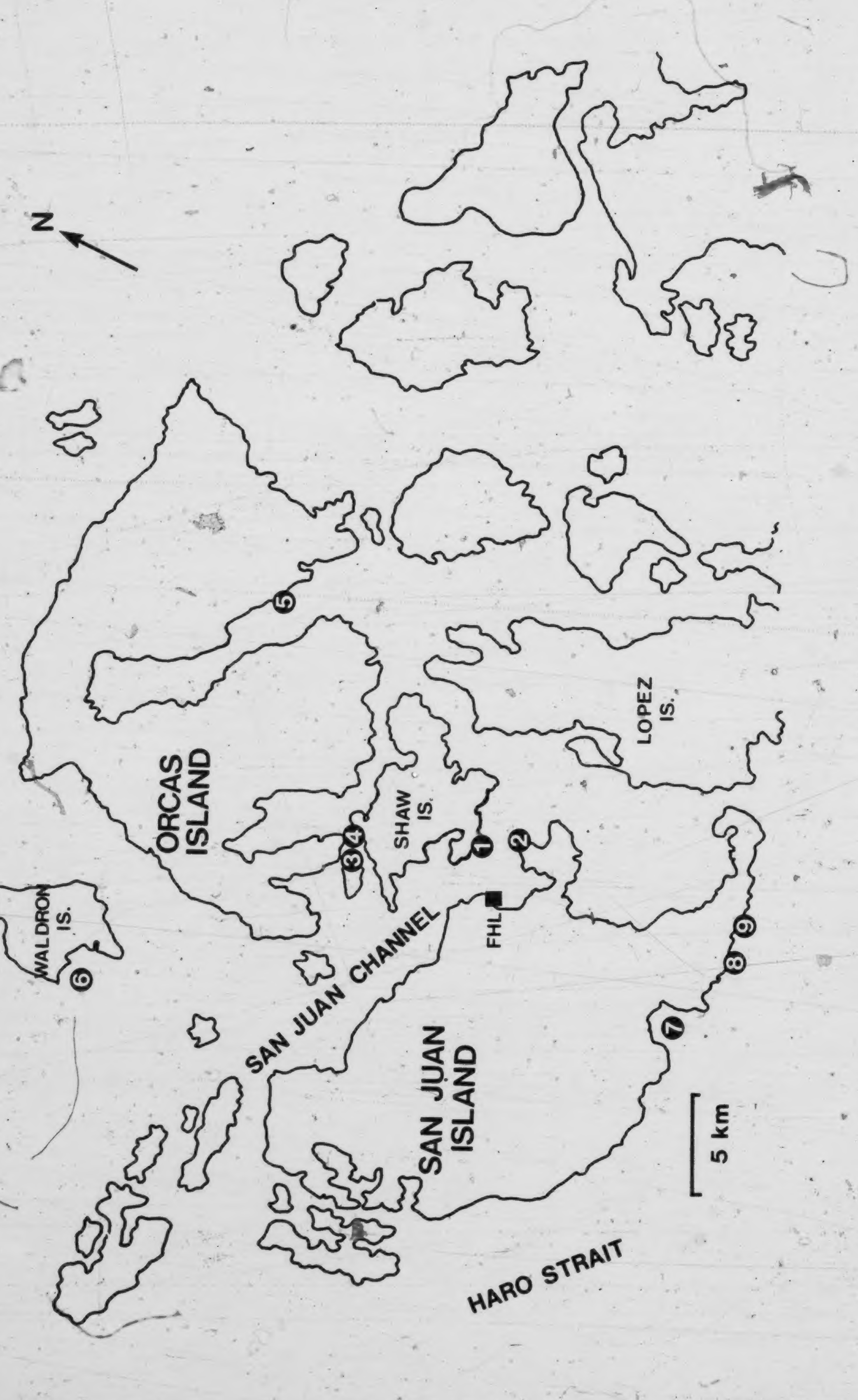
The study of reproductive behavior has its justification in the acknowledgement that behavioral traits have costs and benefits in terms of their effects on reproductive success. Reproductive behaviors are therefore assumed to be intimately related to fitness and to have been under continuous intense selection (Thornhill and Alcock, 1983). Different selective pressures of differing intensities, acting upon species living in a variety of habitats might be expected to result in a complementary set of varied behaviors and morphologies enhancing reproductive success. Each species should respond with morphological adaptations and a behavioral repertoire appropriate to local environmental conditions.

This chapter presents detailed information on the reproductive behavior of 12 species of holothuroids in the San Juan Archipelago, Washington. Behavioral data are related to habitat conditions and species characteristics in an attempt to explain the differences in reproductive activities found among these sea cucumbers. Approximate breeding seasons are also outlined and environmental stimuli inducing spawning events and synchronizing gametogenic cycles are suggested. Holothuroid spawning activities reported to date are reviewed and the reproductive behavior patterns among different taxa are treated. The term spawning is used here as referring to the release of ripe gametes through the gonopore(s) of the adult.

B. MATERIALS AND METHODS

For 10 species of subtidal holothuroids (Cucumaria lubrica, C. miniata, C. piperata, Cucumaria sp., Eupentacta quinquesemita, Molpadia intermedia, Parastichopus californicus, Pentamera populifera, Psolidium bullatum, Psolus chitonoides), collections and diving observations of behavior were made between 0900 - 1700 hrs from August 1981 through August 1983 at six locations in the San Juan Islands, Washington (sites 1 - 6, Fig. II-1). At three sites (False Bay, Eagle Point, and Grandmother's Cove; Fig. II-1), data and specimens of two other species (C. pseudocurata and Leptosynapta clarki) were collected intertidally. For convenience of sampling, habitats with dense populations of holothuroids and in close proximity to the laboratory were chosen as collection sites. Large adults were selected for each species. Information on spawning behavior was also gathered from adults of all 12 species at Friday Harbor Laboratories where they were provided substratum from their natural habitats (mud, sand, or rock) and maintained year-round in a flow-through seawater system. Extremes in seawater temperatures in the holding tanks and seawater tables were 7° C in winter and 14° C in summer. The sea cucumbers were subject to sunlight entering windows on the north and south sides of the laboratory. Breeding seasons were estimated from dive sightings of field spawning, natural laboratory spawnings, qualitative assessments of gonad ripeness, and previous workers' accounts of reproductive activity in the laboratory and occurrence of larvae in the plankton. No attempts were made to record individuals not spawning during mass spawning events. Four categories were used to group gonads of Molpadia intermedia and Parastichopus californicus according to ripeness: unripe, ripe, partially spent, and spent. Microscopical examination was necessary to distinguish between unripe and spent gonads of P. californicus.

Fig. II-1. Major collection and study sites, San Juan Islands, Washington (48° 26 - 43′ N. and 122° 50′ - 123° 10′ W.). 1 - Point George, 2 - Turn Island, 3 - Crane Island, 4 - Bell Island, 5 - East Sound, 6 - Cowlitz Bay, 7 - False Bay, 8 - Eagle Point, 9 - Grandmother's Cove, FHL - Friday Harbor Laboratories.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WALDRON IS.
6
ORCAS ISLAND
5
SHAW IS.
3
4
1
2
FHL
LOPEZ IS.
SAN JUAN CHANNEL
SAN JUAN ISLAND
7
8
9
5 km
HARO STRAIT

C. RESULTS

A listing of the spawning duration of individuals, number of gonopores, gamete colors, and egg buoyancy by species is given in Table II-1. The following descriptive details are based on laboratory observations unless noted otherwise; field confirmations are noted in the text. laboratory observations; behavior observed in the field is noted in the text.

BEHAVIOR

Psolus chitonoides

Males of this species usually precede females in spawning in the laboratory. The male genital papilla is quite variable in shape and size, is located in between the two dorsal-most tentacles or slightly towards the oral opening, and divides into 4 - 19 finger-like branches. Length from the base of the papilla to the branch tips may reach 6 mm. The main trunk of the papilla is engorged to a bulbous state with sperm (inset, Fig. II-2A) during spawning and the sperm exits through the terminal pores of the papillar branches (Fig. II-2A). Tentacle behavior is unusual among males during gamete release in that the tentacles are in a half-contracted state and alternately swab over the genital papilla and buccal membrane. The tentacles mop up the light pink sperm from the papilla and the buccal membrane where the thick streams of sperm have fallen and then lift the sperm out into the water column as a milky suspension. The tentacles are not stuffed into the buccal cavity as in the usual tentacle feeding pattern. Although one male spawned continuously for 7 1/2 hr, the usual spawning duration for males was 1 - 3 hr. In the laboratory, individual males may release sperm on three or four different occasions on the same day or over a period of several days.

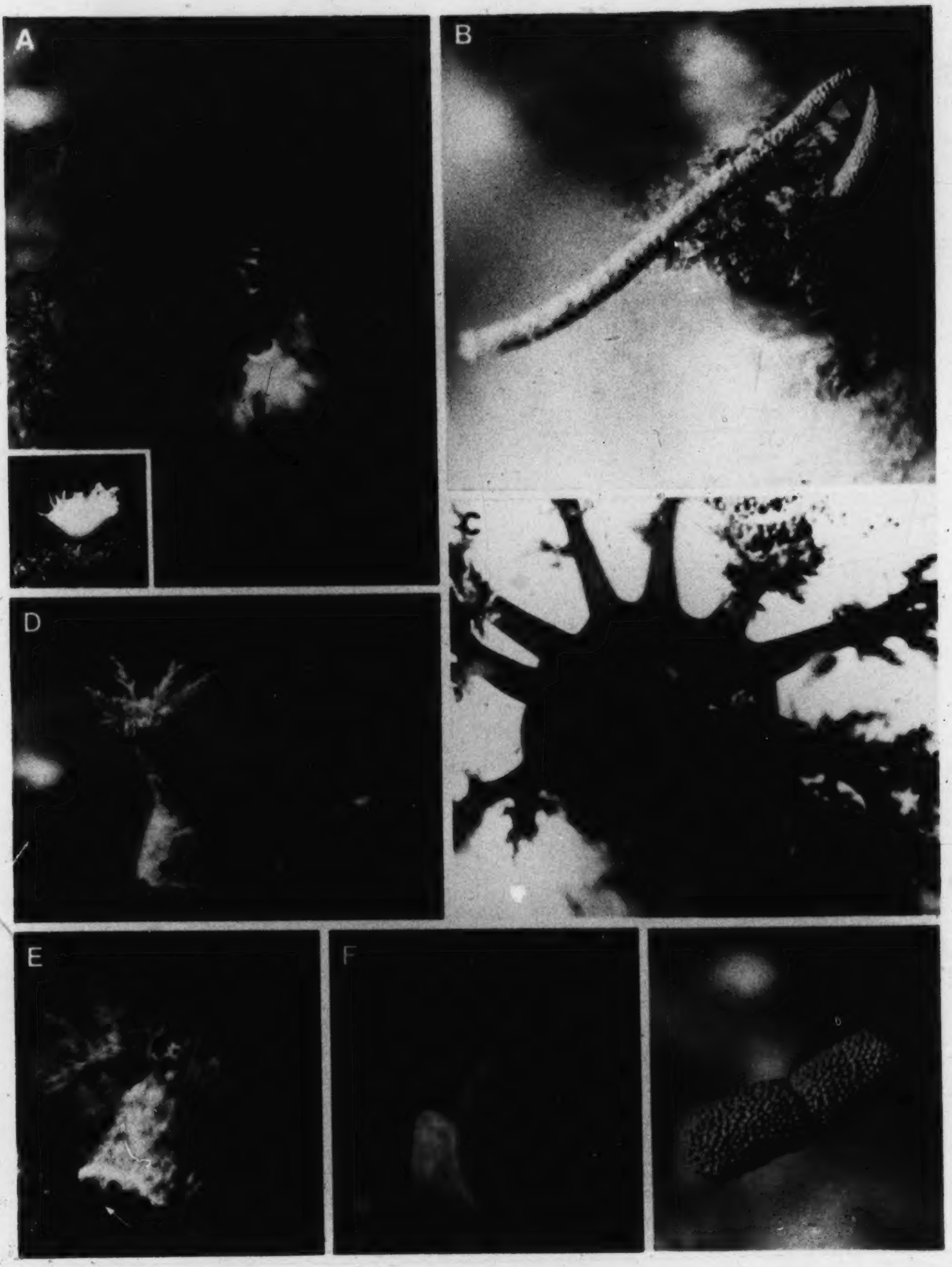
A long, continuous rope of brick-red eggs is produced by females (Fig. II-2B) and has been previously mentioned by other workers (Jones, 1960; Young and Chia, 1982). The egg rope, upon emerging from the single genital pore, has a rounded tip and a width of 2 - 4 mm. The cylinder of eggs gradually narrows in width and leaves the gonopore more slowly towards the end of spawning, which may last up to 90 min. The female genital papilla can be stretched to a length of 8 mm by the egg rope to give the papilla the

Table II-1. Reproductive characteristics of spawning holothuroids and spawned gametes (laboratory data collected at Friday Harbor Laboratories). A 1 in brackets is the gonopore known to be opened at time of egg release. Gamete pigmentation determined under laboratory flourescent lighting; the most common color encountered is underlined. Egg buoyancy symbols: ++ = float at surface soon after release, θ = slightly negatively buoyant, - = negatively buoyant, -- = very negatively buoyant. Three dashes in other columns indicate no data. Numbers in parentheses are numbers of adults observed.

Species	# Gonopores		Spawning duration of individuals in laboratory (hr)		Pigmentation of gametes		Egg buoyancy
	♂	♀	♂	♀	sperm	eggs	
Cucumaria lubrica	1-4 (54)	1 (3)	2+ (17)	1-4 (3)	white	*dull yellow*, tan, light yellow, light green, bright yellow, green	--
C. pseudocurata	1 (60)	1 (5)	1 1/2-4 (20)	---	white	bright orange	--
C. miniata	1-9 (21)	[1] (70)	3-6 (7)	3/4-4 (10)	*white*, orangish pink	*green*, olive green	++
C. piperata	1-7 (12)	[1] (22)	2 (2)	---	white	olive green	++
Cucumaria sp.	5-6 (11)	1 (16)	---	1-2 1/2 ([illegible])	*white*, light	*tan*, brown, olive green	++

					orange		
Eupentacta quinquesemita	---	1 (30)	1 (4)	1-1 3/4 (8)	white, light yellow	light green, olive green	0
Pentamera populifera	1-7 (41)	1 (30)	---	1-2 (4)	white	light green, dull white	0
Psolidium bullatum	---	---	---	1/2-1 (26)	white	golden yellow, brownish orange	0
Psolus chitonoides	4-19 (63)	[1] (105)	1 1/2-7 1/2 (19)	1-1 1/2 (15)	pink, white	brick red	++
Molpadia intermedia	1 (8)	[1] (1)	1/2-3 (7)	3-4 sec (1)	cream	orangish pink	0
Parastichopus californicus	1 (5)	1 (2)	---	---	white	light orange	0
Leptosynapta clarki	---	---	1-2 (2)	---	white	pink, white	-

Fig. II-2. (A) Male Psolus chitonoides releasing sperm from pores at ends of branches of genital papilla; the half-contracted tentacles are actively swabbing over the papilla and over the buccal membrane where the sperm falls. Inset: bulbous genital papilla of another male that is swollen with sperm; small finger-like branches radiate from the papilla. Magnification: 1.3x. (B) Long, cylindrical, egg rope produced by P. chitonoides. (C) Tubular genital papilla (arrow) of P. chitonoides filled with eggs. Magnification: 1.7x. (D) Occasional stance assumed by male Psolidium bullatum, with stretched introvert and half-contracted tentacles. Magnification: 1.8x. (E) Adult P. bullatum just beginning to detach tube feet (arrow) on anterior half of the sole prior to spawning. (F) Normal spawning posture of adult P. bullatum in upright arched position with front part of the sole freed from the substratum. (G) First eggs spawned by Cucumaria miniata packaged in a double pellet. Pellets begin to expand and eggs loosen up 1 - 2 min after being released (as in this photograph). Magnification: 2.5x.


A
B
D
E
F

appearance of a light red, thin sleeve (Fig. II-2C). The positively buoyant eggs are bound together by a transparent, mucoid substance. In the field and laboratory, the rope fragments into sections of varying length that float to the water surface where the mucoid material breaks up or dissolves in 10 - 15 min, releasing the eggs to be dispersed by currents. Only 4 of 145 females in the laboratory were observed using the tentacles to break up the recently extruded egg rope with a behavior similar to males, but with much slower movements. Females are one-time spawners and were never seen to spawn repeatedly unless disturbed; when disturbed during the course of gamete release, tentacles were retracted for 5 - 10 min and then extended again and spawning was resumed.

Psolidium bullatum

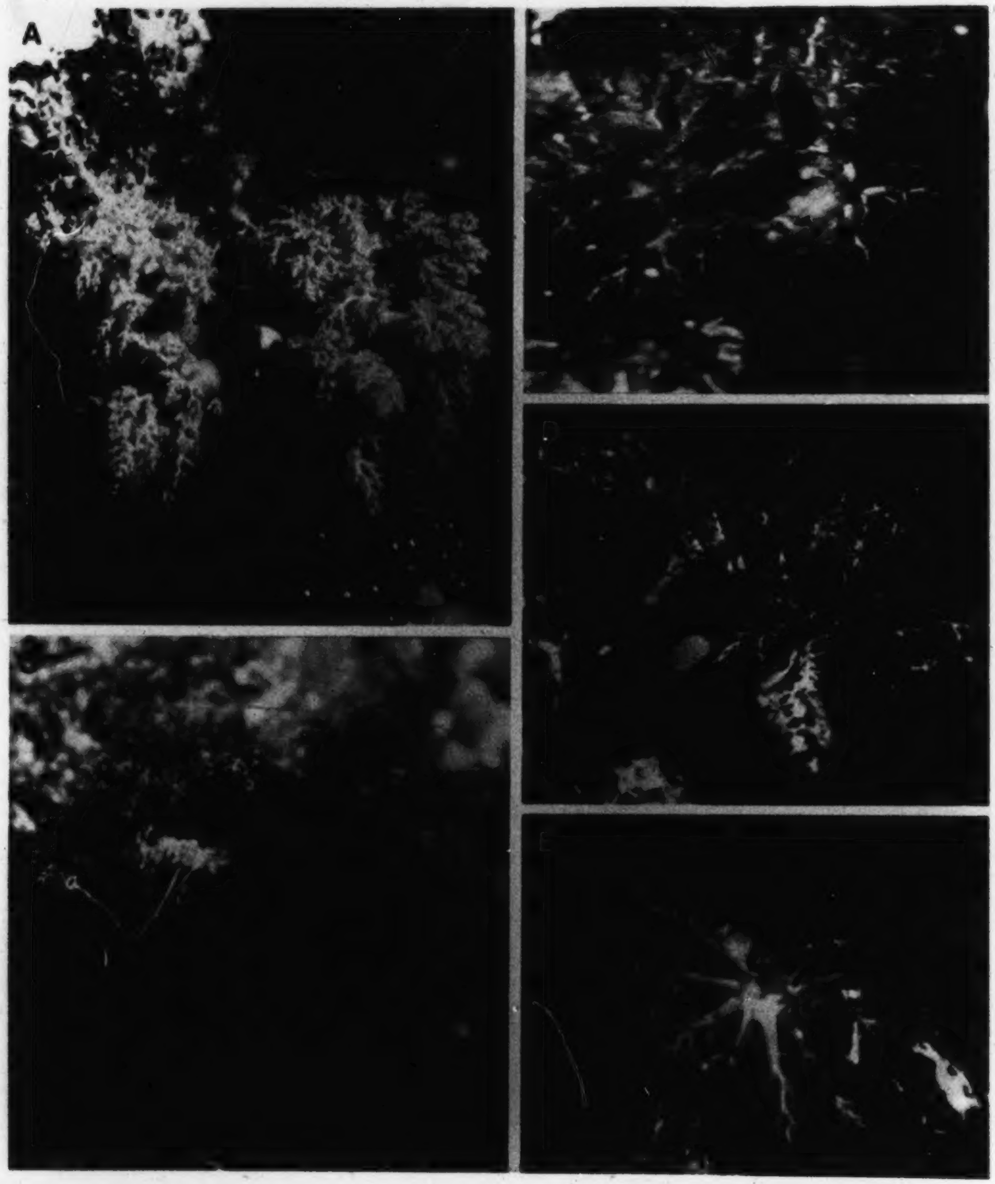
This small psolid (up to 2.5 cm length) has a very low, flat profile of not more than 2 - 3 mm height and is quite sedentary in its habits, rarely moving more than 2 - 3 cm under laboratory conditions. Occasionally, males would remain with the sole attached and extend the introvert (Fig. II-2D) to put out small amounts of sperm. Just prior to spawning, both sexes lift up the anterior half of the sole, detach the tube feet on the rim of the sole (Fig. II-2E), arch back (Fig. II-2F), and then release their gametes; sperm disperses rapidly and males precede females in spawning.

Cucumaria miniata and C. piperata

Eggs of these two cucumariids are tightly packaged in pellets held together by a mucoid substance. The pellets are similar to the egg rope of Psolus chitonoides in that they expand within 1 min after release and then fall apart after 15 - 20 min. The first few pellets of C. miniata are often produced in pairs (Fig. II-2G) with successive pellets being single; large adults produce 20 or more of these pellets.

As eggs are collected in the gonoduct of C. miniata, the first pellet is pushed up into the very end of the genital papilla at the base of the tentacles. The genital papilla of one adult was positioned on the introvert, 1 cm posterior to the tentacles. There is no gonopore in the females, so that the papilla is ballooned by the pellet (Fig. II-3A) and the epidermis is stretched. The thin layer of stretched epidermis at this point reveals a seam

Fig. II-3. (A) Field photograph (Point George) of the greatly expanded genital papilla of Cucumaria miniata, prior to release of the first egg pellet. The epidermis has been stretched thin by the pellet (eggs are distinguishable) and a seam of scar tissue (arrow) from previous years' spawnings is visible. Magnification: 1.2x. (B) Newly spawned egg pellet of C. miniata and the recently opened genital pore (arrowhead) from which the pellet has just exited. (C) Streams of sperm falling from gonopores of a male C. miniata. Magnification: 1.1x. (D) Cucumaria piperata spawning an egg pellet at Crane Island. Magnification: 1.2x. (E) Strand of eggs produced by Cucumaria sp. Magnification: 0.8x.


A

of scar tissue from spawnings of previous years (Fig. II-3A). The pellet is gradually forced further into the papilla, causing the epidermal tissue at the tip of the papilla to tear slowly, and the pellet escapes (Fig. II-3B). This process of filling the papilla with eggs and then tearing of the papilla was also noted for Psolus chitonoides and C. piperata.

While still in the act of broadcasting egg pellets, a female C. miniata was dissected for examination of the gonads and gonoduct. The female was in the latter stages of gamete release, but continued to spawn after being opened. The ovarian tubules were completely emptied of eggs; a third of the gonoduct length (immediately anterior to the gonadal basis) was expanded to a width of 4 - 5 mm and packed with eggs. Throughout the rest of the gonoduct, four pulses (or pellets) of eggs were being slowly moved towards the gonopore, probably by muscular peristaltic contractions or by the cilia of the gonoduct wall, or a combination of both. Packets of eggs up to 4 cm long leave the genital papilla every 4 - 10 min; subsequent packets are progressively smaller in size (10 - 30 mm length and 3 - 6 mm diameter). Cucumaria piperata egg pellets (Fig. II-3D) are smaller (8 - 23 mm length and 2 - 3 mm diameter) with slightly tapered ends and are let loose at 2 - 5 min intervals.

Observations in the field reveal that reproductive behavior is very similar to laboratory behavior: both sexes stretch the anterior third or more of the body out from underneath rocks and up into the water, often clinging to the sides of the rocks with their podia. Tentacles are held at a slight upward angle in a half-contracted, motionless state. When water circulation was turned off in laboratory tanks, a slow, back-and-forth swaying movement was exhibited by two male C. miniata in the laboratory; one complete cycle of swaying lasted approximately 40 - 90 sec. Male spawning preceded that of females. Females in the laboratory and all females watched in the field were quiescent while putting out egg pellets, but one female in the laboratory (when water circulation was cut off in the tank) swayed relatively rapidly (9 - 10 sec for one back-and-forth cycle) and moved the tentacles slightly, which did not contact the pellets. Ambulacral podia on the introvert region posterior to the tentacles of males of both species are elongated in the course of sperm emission and actively move about in a waving fashion, apparently as an aid to dispersal of gametes. Thick streams of sperm are emitted by males of both species, which, in the laboratory, frequently fell and collected on the bottom of the holding tanks.

No instance of sperm accumulation was ever witnessed in field spawnings; sperm was readily carried up by weak currents and quickly diluted within 5 - 15 cm from the genital papilla (Fig. II-3C).

Cucumaria sp.

Small individuals of Cucumaria sp. put out short, irregular, narrow segments of eggs with tapered ends; larger adults produce a larger, long, continuous strand which is more uniform in size (Fig. II-3E). These strands are occasionally 2 - 4 eggs wide (1.0 - 2.0 mm diameter), but more often are 1 - 2 eggs wide and are easily reduced to short sections by currents. Individual eggs become separated from each other in 12 - 15 min. Tentacles sometimes aid in fragmenting the egg strands whereas male tentacles are held stationary at a 45° angle during sperm emission. Tentacle posture among females is variable: tentacles are ordinarily half-contracted in a lateral position (as in Fig. II-3E), but may also extend vertically in a more contracted and bushy appearance.

Eupentacta quinquesemita

Spawning details for this holothuroid are based solely on recorded laboratory behavior, since field spawnings were never observed. Eupentacta quinquesemita lives on the upper side of large boulders and on rock walls in areas of high current. Eggs and sperm in the gonoducts are visible through the thin wall of the introvert; sperm are transported through the gonoduct in rhythmic pulses, whereas eggs leave the gonopore in a steady stream and then in pulses in the last stages of spawning. A sticky mucus surrounds the eggs, forming a loose rope that falls into a pile at the bottom of the dish. In seawater tables with steady current flow, these loosely-held eggs are quickly separated and scattered within 1 - 2 min. Some females deposited the eggs on the buccal membrane and demonstrated both pronounced tentacle and swaying motions; when eggs fell to the side of the individual and not on the buccal membrane, only very slight, slow swaying was noticeable with no tentacle movement.

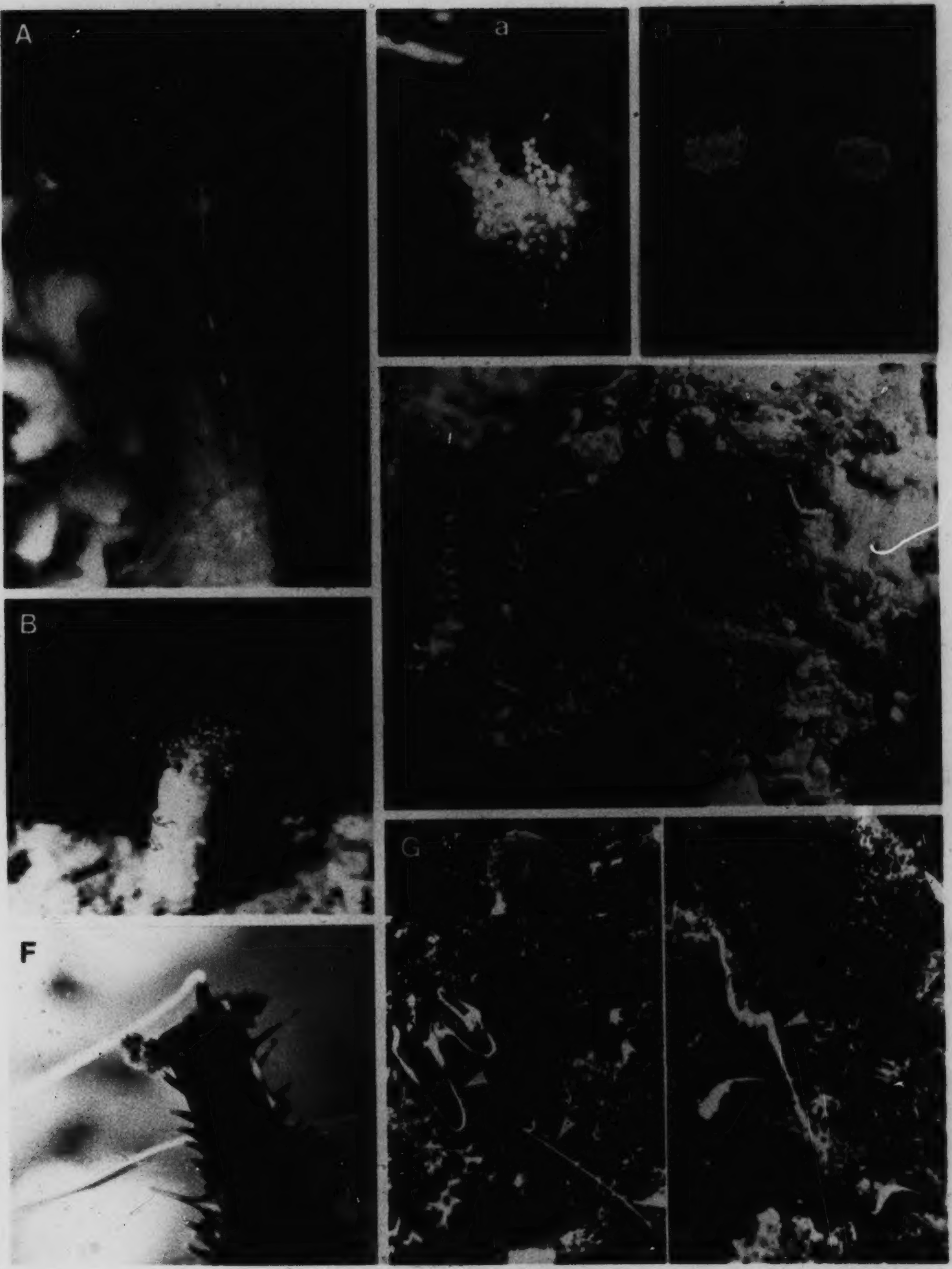
Pentamera populifera

Pentamera populifera extends its long introvert out of the mud substratum in which it lives and reaches high up to release gametes. Male tentacles are commonly held out to the side in tightly contracted bunches and only rarely swab the papilla. Passage of one pulse of sperm within the gonoduct from gonad (located midway along the main body axis) to gonopore takes 2 3/4 - 3 min and as many as five sperm pulses are present at one time in the spermatic duct (Fig. II-4A). Tentacles of females are not as tightly contracted and are bent over to ensnare the loose eggs falling on the buccal membrane and afterwards swing out to the side to cast the eggs into the slow current (Fig. II-4B). Field spawners did not catch all the eggs and many eggs fell out of the gonopore off to the side, being either carried away by currents or landing on the mud surface and moved along on top of the mud.

Cucumaria lubrica

Although the brooding behavior of C. lubrica has been described previously by Engstrom (1974, 1982), additions and differences seen in the spawning behavior of this species are treated here. Females spawn 2 - 7 hr after males and, without losing their grip on the hard substratum, tuck the anterior quarter or third of the body directly underneath the venter for egg deposition (Fig. II-4C). They may remain in this folded condition 1/2 - 2 hr before eggs are released. At the close of egg-laying, females occasionally detached anterior podia on one side of the trivium to allow the tucked in buccal region to withdraw. At this time, females on vertical sides of culture bowls were particularly vulnerable to egg loss. Engstrom (1974, 1982) also witnessed many eggs of this species not being caught during spawning and calculated that 37.9 % were missed by females in the laboratory. This whole process of egg deposition was witnessed in 15 - 18 females in the laboratory; 3 - 4 females were observed in the field in the same humped posture during heavy spawning of males. Engstrom (1974, 1982) says that two of the three ventral rows of ambulacral podia release hold of the substratum and the female fills the ventral surface with eggs from posterior to anterior and then re-attaches the podia to pin the eggs between the body and substratum. Though this procedure was never observed by me, C. lubrica may possess more than one method of egg-laying.

Fig. II-4. (A) Three pulses of sperm (arrows) moving up gonoduct of Pentamera populifera. Magnification: 1.6x. (B) Female individual of P. populifera dispersing eggs by moving tentacles over the region of the genital papilla and buccal membrane, ensnaring the eggs, and then lifting them out into the water current. (C) Ventral view through bottom of glass dish of Cucumaria lubrica at onset of egg laying with anterior end (a) tucked under the trivium. Arrow points to eggs just leaving the gonopore. Magnification: 1.8x. (D) Sperm bundles of C. lubrica with tails of the spermatozoa intertwined. Magnification: 275x. (E) Spawning male of C. lubrica (approximately lifesize) at Turn Island in December with anterior half of body elevated, issuing forth a thick stream of sperm that is wafted away by water currents. (F) Three-mm long genital papilla of C. lubrica pouring out a stream of sperm, with contracted tentacles held laterally. Magnification: 2x. (G) Heavy streams of sperm (arrows) caught among members of population of C. lubrica at Turn Island during field spawning.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a
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G

Engstrom also noted that males arched the oral end of their bodies up away from the substratum and produced large, heavy streams of sperm (Fig. II-4E,F) that are wafted away by currents to later lodge against other members of the population (Fig. II-4G). Observed under low power on a dissecting microscope, sperm can be seen exiting the gonopore in bundles (Fig. II-4D). These bundles were first mentioned by Atwood and Chia (1974) and are, to my knowledge, the only spermatophore-like structure known among echinoderms. Under laboratory conditions, males displayed errant behavior, moving around and climbing to the tops of culture bowls, rocks, and the sides of the seawater tables where they would spawn. This wandering behavior was also noticed among males in the field during winter months. A firm attachment to the substratum was maintained by the sedentary females, even prior to the onset of brooding.

Cucumaria pseudocurata

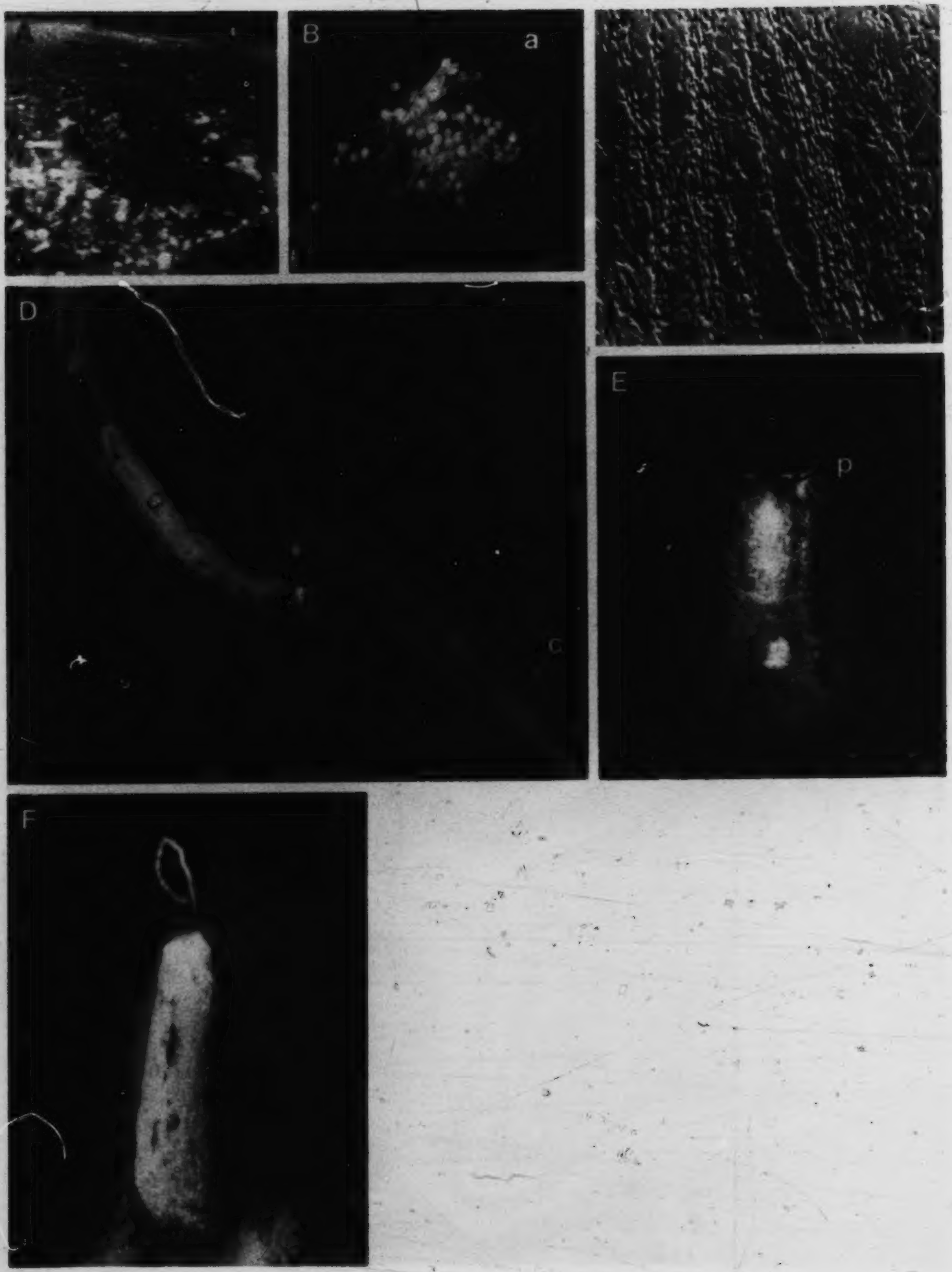
This small dendrochirotid is a brooder (Clark, 1901a,b; Smith, 1962; Rutherford, 1973), inhabiting intertidal beds of Mytilus californianus. Behavior in both sexes is quite similar to that of C. lubrica, with slight differences. Just before the oral end is tucked under, females reach out further than is usual with the podia and cling tenaciously to the substratum (Fig. II-5B). This apparently results not only in reducing chances of egg loss, but also increases surface area available for brooding. Brooding individuals can be detected by their more flattened, low profile and extended tube feet (Fig. II-5A).

Podia are normally left attached and only the introvert and buccal area are turned up for spawning in males. The long strings of sperm produced are quite resistant to dilution and can be picked up with forceps. Spermatozoa are aligned in strands along the long axis of the sperm string (Fig. II-5C). According to Atwood (1975a,b), these strands are composed of mucus.

Cucumaria pseudocurata may also have an alternate way of placing the eggs underneath the trivium for brooding. In northern California, females of C. pseudocurata "arch their expanded tentacles to catch the extruded eggs. These eggs are then transferred to the ventral surface and arranged by the extensile podia..." (Smith, 1962).

Eggs are extremely sticky and cling together in one mass. Large quantities of mucous secretions are reported to cover the body (Smith, 1962) and egg masses

Fig. II-5. (A) Dorsal view of female *Cucumaria pseudocurata* during egg deposition. Anterior end (a) is tucked under and lateral tube feet grip substratum, flattening out the body. Magnification: 2x. (B) Ventral view (through glass dish) of female *C. pseudocurata* laying eggs underneath trivium, similar to *C. lubrica* (Fig. II-4C). Anterior end (a) is tucked underneath. (C) Strand of sperm of *C. pseudocurata* compressed between microscope slide and coverslip, showing the longitudinal arrangement of the tabloid-shaped spermatozoa. Magnification: 325x. (D) Pair of spawning male *Leptosynapta clarki*. Arrows indicate white dots where sperm is leaving the gonopore. The male on the right is terminating its spawning whereas the male to the left is just beginning to spawn, evidenced by the full, ripe, white testes visible through the body wall of the latter. Body wall contractions (c) pass up and down the length of the male during gamete release. Magnification: 1.5x. (E) Upright stance of a female *Molpadia intermedia*, 2 - 3 min before spawning, with a broad, conical, genital papilla (p). The swollen zone (white area) below the papilla is epidermis stretched thin by the underlying gonoduct containing eggs collected from the ovaries. m is the madreporite. Magnification: 1.1x. (F) Male *M. intermedia* spewing out sperm. Magnification: 0.7x.


A
B
a
D
c
E
p
F

(Atwood, 1875a,b), but I found only one brooding female in the field to be covered by an unusual amount of mucus (observations made throughout four consecutive breeding seasons).

Parastichopus californicus

Typical of most aspidochirotids, both sexes of P. californicus rear up the front half of the body to broadcast eggs and sperm. Gametes are slowly dribbled out of the small conical papilla while the individual sways slowly from side to side. Pseudocopulation has been observed in the field and involves paired adults in an upright posture wrapped around each other during the act of spawning (J. Elliott, personal communication).

Leptosynapta clarki

During middle and late November, adults can be collected from muddy sand in False Bay in small groups of 2 - 4 entwined around one another, or side by side with their oral ends close to or flush with the surface of the sand (also Everingham, 1961). In False Bay in each of the breeding seasons of 1981 and 1982, water was collected by pipette from 10 of the small depressions formed in the sand by the synaptid aggregations, but no spermatozoa were ever found. Sex ratios for these aggregations were not determined, but male:female ratios on 9 December 1982 (67 adults) and 29 October 1983 (102 adults) were 2.9:1.0 and 2.3:1.0, respectively. Free-spawning of males occurred on one occasion in the laboratory: a small dot of white sperm was visible in between and slightly posterior to the bases of two tentacles (Fig. II-5D). Waves of strong contraction passed up and down the body wall, accompanied by active tentacle movements.

Molpadia intermedia

This large, slow-moving molpadiid travels through and ingests the fine sediments in which it is buried; the only time adults of this species are known to surface is for spawning. Rising 4 - 8 cm straight out of the mud, males emit sperm over a period of 1 1/2 - 3 hr. The short digitate tentacles are retracted well into the buccal region. The dorsal genital pore lies 1.0 - 1.5 cm behind the tentacles; a temporary genital papilla in the shape of a small cone-like protruberance is formed and is the most prominent feature during spawning activity. A puff of sperm is spurted 3 - 5 cm high into the water column above the genital papilla (Fig. II-5F) at intervals ranging from 0.7 - 6.5 min (n=16), with pauses of 1.5 - 2.0 min most common in between the 1 - 2 sec-long emissions.

A perpendicular position was also assumed by the single female which spawned in the laboratory (Fig. II-5E). This motionless stance was maintained for 1 1/2 - 2 hr while eggs were collected from the ovaries into the gonoduct. Evidence for this was a slow swelling of the genital papilla (a squat cone shape, seen only during reproductive behavior) and particularly of the region of the body wall from the papilla down to 2 cm below the papilla (Fig. II-5E). A pink color gradually appeared simultaneously with growth of the swelling, due to the orangish-pink pigmentation of accumulated eggs in the gonoduct showing through the thinly stretched body wall. Eggs are spewed 12 - 15 cm into the water above the female in one explosive burst lasting 3 - 4 sec, after which the female retracts into the mud and does not spawn again.

In the middle of August, five adults were relaxed in a saturated solution of magnesium chloride in seawater for 5 hr and then dissected to examine the gonoduct and gonopore for tissue barriers that might prevent exit of gametes through the gonopore. Red dye was injected into the gonoducts of the three females which caused the gonoducts to balloon. Further dissection revealed no detectable gonopore present in the females - only a continuous layer of tissue at the gonoduct/body wall junction. Injected dye exited readily from the gonopores of the two males and there was no inflation of the thinner-walled male gonoducts.

SPAWNING IN THE FIELD AND SPAWNING STIMULUS

Of the 27 separate occasions when spawning was sighted in the field (Table II-2), observations of only females spawning were restricted to two cases (three individuals of Cucumaria miniata and a single Psolus chitonoides), whereas males (with no females) were seen emitting gametes on 11 dives. Mass population spawnings of males were witnessed twice for C. lubrica and once for Pentamera populifera and P. chitonoides.

Synchronous, multi-species, mass spawnings took place at three sites during a 2-week period in 1983. On March 24, the Cucumaria miniata population at Bell Island was spawning, along with the two asteroids Pycnopodia helianthoides and Solaster dawsoni.

Table II-2. Laboratory and field observations of holothuroid spawning, May 1981 - August 1983, San Juan Archipelago, Washington. Numbers in parentheses are field sightings of egg pellets, separate from in situ spawning observations of adults; p is for population spawning. Field observations for *Cucumaria pseudocurata* and *Leptosynapta clarki* were made during intertidal collections at low tide series. Number of large adults maintained year-round in sea water tanks: 10 - 20 for all dendrochirotid species and 5 - 10 *Parastichopus californicus*. Ten to fifteen *Molpadia intermedia* adults were kept in the laboratory from September through February and 20 - 30 adults of *Leptosynapta clarki* were maintained from October through April.

	LABORATORY SPAWNING (cumulative total)			FIELD SPAWNING				
Species	**# Observed**		**Date(month)**	**Site**	**# Dives when spawning observed/ # dives at site**	**Date(month)**	**# Spawning/dive**	
	♂	♀					♂	♀
Cucumaria lubrica	159	67	Dec,Jan	Pt. George	1/63	Dec	p	0
				Turn Island	2/22	Nov,Dec	p/p	0/4
C. pseudocurata	129	45	Dec,Jan	Eagle Pt. (intertidal)	0/16	---	0	0
C. miniata	90	77	Mar-May	Pt. George	2(1')/63	Apr,May	p/2	p/1
				Turn Island	3'(1)/22	Apr,May	1/0/5	0/3/4
				Bell Island	3(2)/19	Mar	6/p/p	0/p/p
				Crane Island	2'(1)/8	Mar	p/p	p/p
				One Mile Reef	1'	Mar	p	p
C. piperata	44	32	Feb-May	Pt. George	2/63	Mar,Apr	1/p	0/p
				Crane Island	2'/8	Mar	p/p	p/p
Cucumaria sp.	11	17	Mar-May	Pt. George	1/63	Apr	p	p

Eupentacta quinquesemita	82	60	May-June	Turn Island Bell Island	0/22 0/19	--- ---	0 0	0 0
Pentamera populifera	116	53	Feb-May	Cowlitz Bay	6/21	Feb-May	p/2/7/ 15/25/2	0/0/1/ 6/7/0
Psolidium bullatum	51	55	Mar-May	(same as for P. chitonoides)	0	---	0	0
Psolus chitonoides	221	145	Mar-June	Pt. George Turn Island Bell Island Crane Island Edward's Reef	1/63 1[1]/22 1[2]/19 1/8 1/1	July Mar May May Apr	p 1 "many" 0 1	p 0 0 1 0
Molpadia intermedia	8	1	Dec	East Sound	0/24	---	0	0
Parastichopus californicus	5	2	Apr,May, July	Deadman's Bay	1[3]/3	May	4-6	3-4
Leptosynapta clarki	2	0	Oct,Nov	False Bay (intertidal)	(aggrega- tions)/22	--- (Oct,Nov)	0	0

Total # species observed spawning in field: 7

# of dives when in situ spawning observed: 27

Total # dives, May 1981 - August 1983: 223

[1] - one observation at this site by R. Shimek (pers. comm.)

[2] - one observation at this site by D. Nickens (pers. comm.)

[3] - one observation at this site by G. Paulay (pers. comm.)

Heavy spawning activity in nearby sympatric populations of *C. miniata* and *C. piperata* on the east side of Crane Island (600 - 700 m west of Bell Island) occurred March 25 and 26. These two species and *Cucumaria* sp. also spawned simultaneously at Point George on April 6.

With two exceptions, the 27 field sightings of holothuroids releasing gametes were during hours of sunshine (not cloudy weather). The two exceptions were populations of *Cucumaria lubrica* at Turn Island, and *C. miniata* and *C. piperata* at Crane Island; at the latter site, heavy spawning had already begun at least one day prior (on a sunny day). In Barkley Sound on the west coast of Vancouver Island, *C. miniata* populations have been seen spawning synchronously on sunny days and also during cloudy weather after two days of sun in the first week of April (R. Shimek, personal communication). The epidemic spawning in the Bell Island-Crane Island vicinity developed at the end of a 12-day stretch of sunny weather, during a dense bloom of phytoplankton that had started 1 1/2 - 2 weeks before. *Cucumaria miniata* at One Mile Reef underwent a substantial bout of spawning on a sunny day (March 30; D. Nickens, personal communication) during this phytoplankton bloom, but with only partly sunny weather the day before. The April 6 spawning of three dendrochirotid species at Point George took place on the fourth consecutive day of bright sun. In Cowlitz Bay, reproductive behavior was seen among members of the male population of *P. populifera* on February 22, 1983, after two days of sunny weather. In a winter-breeding species, *Cucumaria lubrica*, there were three and four days of sun prior to populations spawning on 7 and 8 December, 1982, at Point George and Turn Island, respectively. The above observations might suggest that spawning may be associated with sunny weather or phytoplankton blooms, but no meteorological data nor measurements of phytoplankton production were recorded on days when no spawning was witnessed. Therefore, these patterns could have resulted from a biased choice of diving conditions.

There are no data for epidemic field spawning of *Parastichopus californicus*, except for individuals seen at Deadman's Bay (G. Paulay, pers. comm.; Table II-2) in the last week of May, 1983, during a 12-day spell of sun. Heavy spawning in this species also took place the same week in the laboratory on May 29 (the 12th consecutive sunny day) and also on May 28, 1981 (on the third day of sun). An almost annual event at Friday

Harbor Laboratories is a mass spawning of numerous marine invertebrates (including P. californicus) on a single day in the latter half of May, usually the last week. This mass release of gametes happens in late evening hours (2100 - 2400 hrs) following successive days of sunny weather and increasing air temperatures.

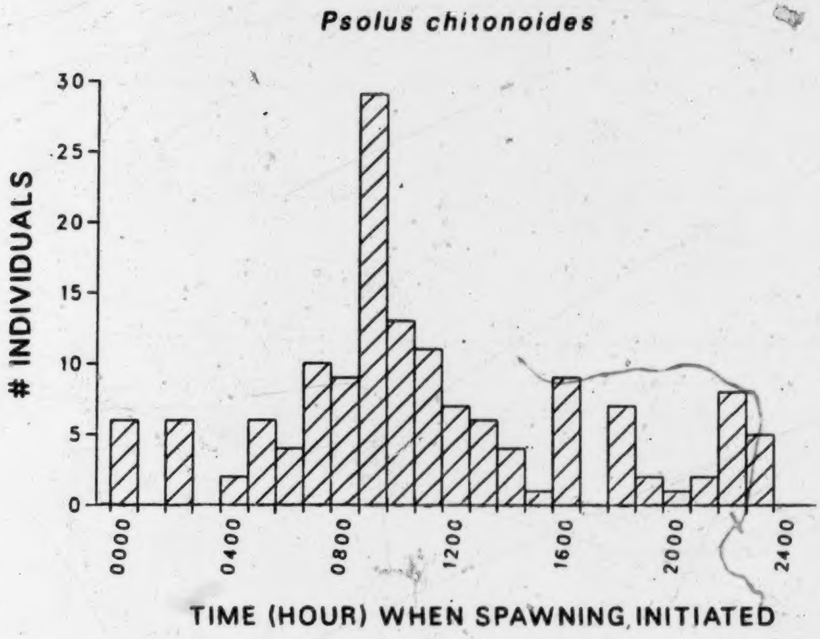
TIME OF GAMETE RELEASE

Time of day when spontaneous gamete release began in the laboratory was recorded for four species (Figs. II-6, 7): Psolus chitonoides, Cucumaria miniata, Eupentacta quinquesemita, and C. lubrica.

Psolus chitonoides spawned at any hour of the day, with heaviest spawning in the morning (Fig. II-6). This psolid is one of the few species in which ripe individuals could, with facility, be induced to spawn. On sunny days, gametes for embryo cultures were obtained by exposing seawater tables holding ripe adults to sunlight, which caused spawning to commence within half an hour. Spawning could be delayed 1 - 2 months by maintaining ripe individuals in dark, seawater tanks. On two separate occasions an all-night vigil was kept (March 1983) on ripe adults collected the day before; no reproductive activity was noted until 15 - 20 min before sunrise when there was an increase in light intensity. In these two instances and when seawater tables were exposed to direct sunlight, no change in seawater temperature was detected. In the field, free-spawning in the population at Point George had already commenced when observed at 1000 - 1100 hrs. Four females were noted releasing the last of their eggs, indicating that females had initiated egg release at least 1 - 1 1/2 hr earlier (0830 - 0900 hrs) and males possibly as early as 0730 - 0800 hrs. A solitary female at Pole Pass was seen spawning at 1500 hrs.

Time of day when reproductive activity occurs in the field is also in general agreement with laboratory observations for Cucumaria miniata. Free-spawning populations were observed at 0930, 1000, 1300, and 1400 hrs at Bell Island, Crane Island, and Point George; 77% of all laboratory spawnings took place in the late morning into the afternoon between 1000 - 1700 hrs (Fig. II-6). R. Shimek (personal communication) has witnessed spawning in this species to commence at 0915 - 0930 hrs at Helby Island (west side of Vancouver Island) and gamete release in a population at Dixon Island at 1145 - 1230 hrs. Both C. piperata and Cucumaria sp. emitted gametes concurrently with C. miniata in the

Fig. II-6. Distributions of time of day when spontaneous spawning was initiated in laboratory tanks (Friday Harbor Laboratories) by individuals of Psolus chitonoides (148 individuals spawning) and Cucumaria miniata (129 individuals spawning), from May 1981 through August 1983. Both sexes included together.


Psolus chitonoides
30
25
20
15
10
5
0
# INDIVIDUALS
0000
0400
0800
1200
1600
2000
2400
TIME (HOUR) WHEN SPAWNING INITIATED

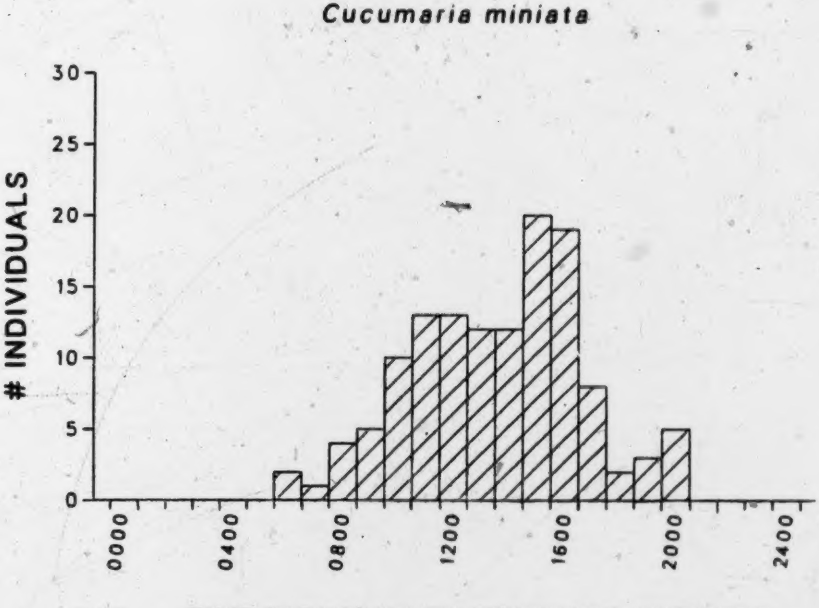
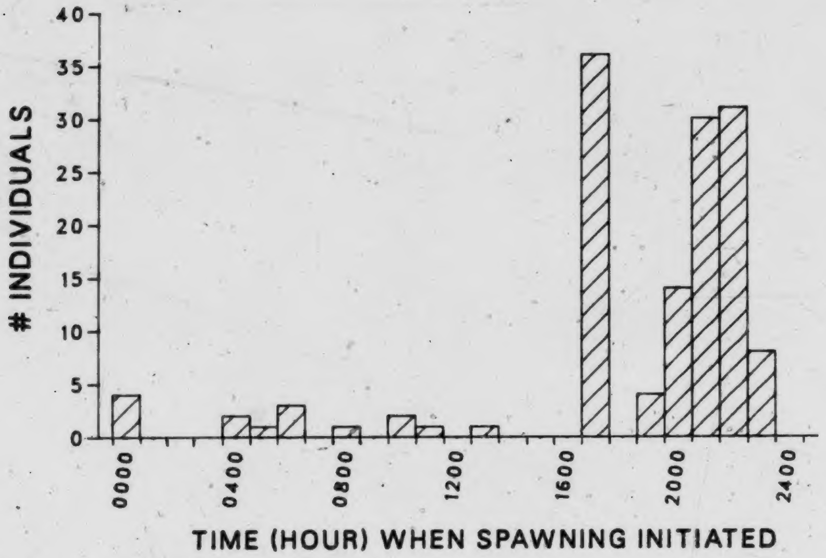
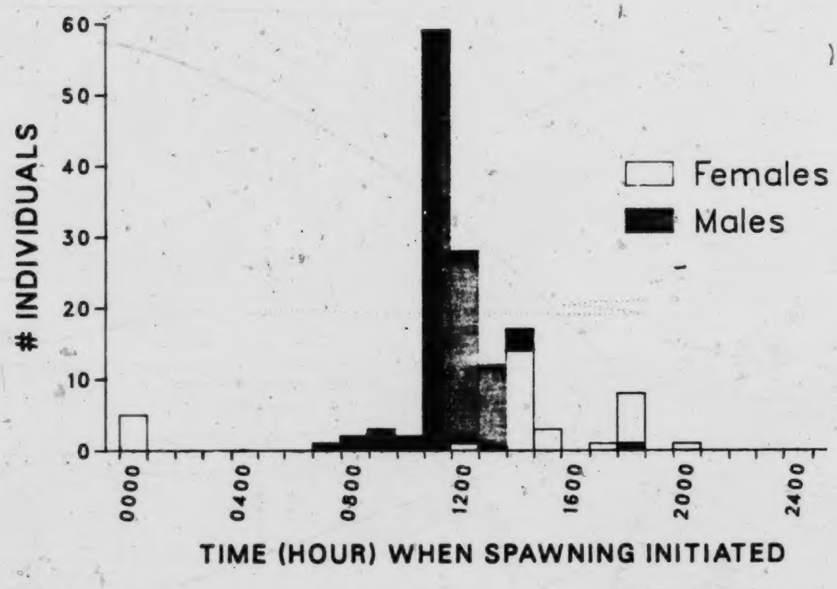

Cucumaria miniata
30
25
20
15
10
5
0
# INDIVIDUALS
0000
0400
0800
1200
1600
2000
2400
TIME (HOUR) WHEN SPAWNING INITIATED

Fig. II-7. Distributions of time of day when spontaneous spawning was initiated in laboratory tanks (Friday Harbor Laboratories) by individuals of Eupentacta quinquesemita (138 individuals spawning) and Cucumaria lubrica (158 individuals spawning), from May 1981 through August 1983. Spawning times of males and females are separated for C. lubrica.

Eupentacta quinquesemita



Cucumaria lubrica



field and laboratory.

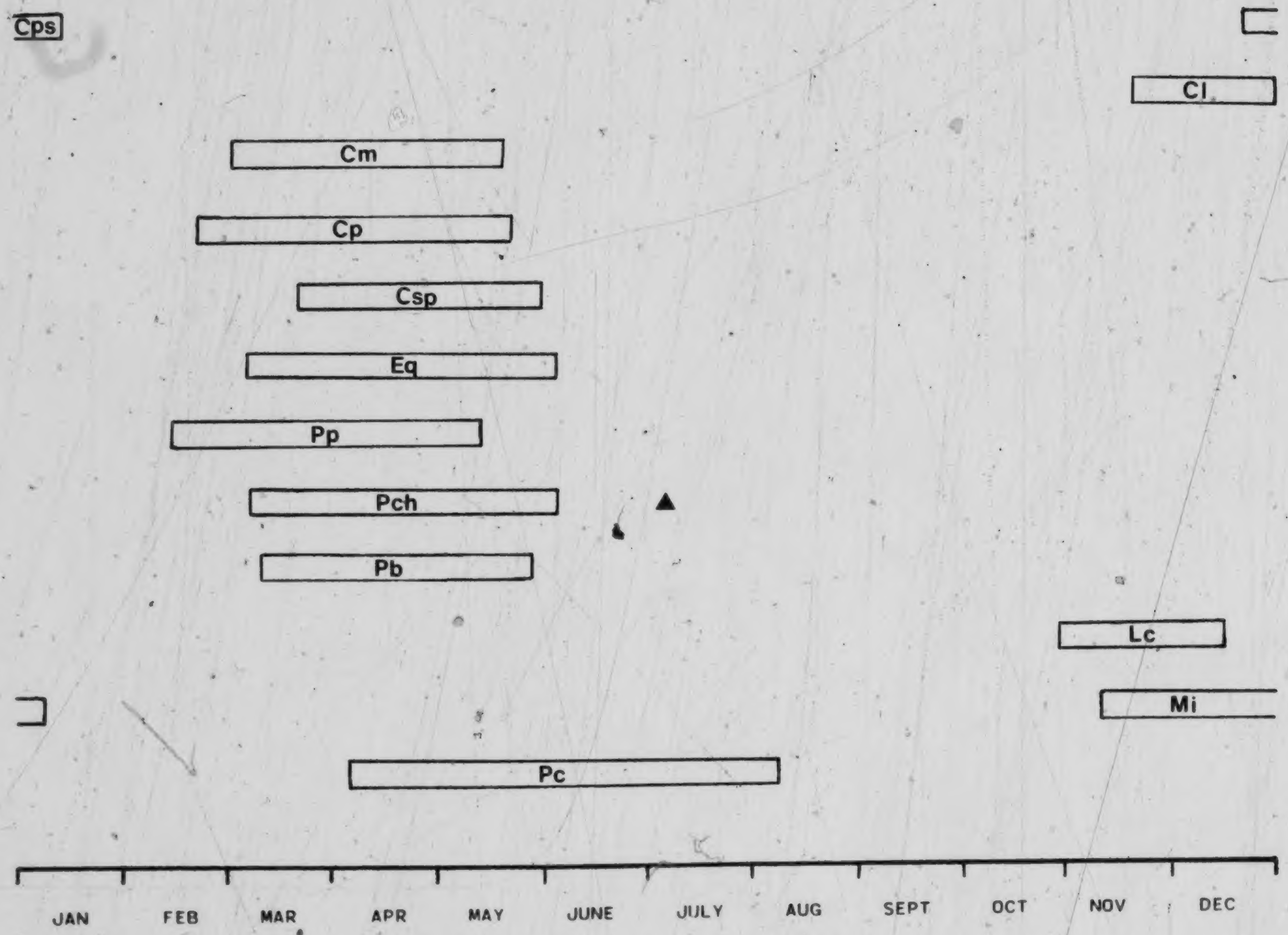
In contrast to the early morning and afternoon behaviors of the psolids and cucumariids, 89% of spawning Eupentacta quinquesemita observed in the laboratory released eggs and sperm in the late afternoon and late evening between 1700 and 2400 hrs (Fig. II-7). If the timing of laboratory behavior is a reliable indicator of when field behavior occurs, E. quinquesemita was not seen spawning in the field because there were no dives during this 7-hour period (1700 - 2400 hrs). Distributions for hour of spawning for Psolus chitonoides, Cucumaria miniata, and E. quinquesemita are all significantly different from each other at $p < 0.01$ (median test, SPSSX, Version 2).

Laboratory data collected for the staggered spawning times of male and female Cucumaria lubrica are similar to data presented by Engstrom (1982). Engstrom (1982) witnessed 100 male C. lubrica spawning between 1130 and 1400 hrs.; 93% (116 out of 125) of the males I observed spawned during the interval 1100 - 1500 hrs. For female C. lubrica, 97% (32 of 33) began egg laying from 1400 - 0100 hrs, whereas Engstrom (1982) saw "several" females laying eggs at 1630 - 1800 hrs. The distribution of time of day when females of C. lubrica commenced spawning (Fig. II-7) is different from that of males ($p < 0.01$, median test, SPSSX, Version 2)).

BREEDING SEASONS

Figure II-8 displays the breeding seasons as approximated from my observations of field and laboratory spawnings (Table II-2) over a 28-month period, estimates of gonad ripeness (% individuals with spent gonads; see text, Table II-3, and Fig. II-10), and presence of embryos and larvae in the plankton. Figure II-9 is breeding seasons based on prior reports from laboratory investigations and plankton hauls. With the exception of Parastichopus californicus, information on the availability of ripe gametes given by Bovard and Osterud (1918) for species collected from the San Juan Islands was not used because of species misidentifications. And due to the difficulty of distinguishing many of the different species' eggs and larvae by color, size, or ciliation pattern, much of the data provided by Johnson (1931) and Johnson and Johnson (1950) were not included. Since Cucumaria curata and C. pseudocurata are now considered the same species (Lambert, 1985), references to these two species are combined under C. pseudocurata.

Fig. II-8. Breeding seasons of 12 abundant holothuroid species in the San Juan Islands, Washington. The period of breeding for each species is based on information from spawning in the field and laboratory (Table II-2) and ripe gonads (see text, Table II-3, and Fig. II-10). Code: Cps - Cucumaria pseudocurata, Cl - C. lubrica, Cm - C. miniata, Cp - C. piperata, Csp - Cucumaria sp., Eq - Eupentacta quinquesemita, Pp - Pentamera populifera, Pch - Psolus chitonoides, Pb - Psolidium bullatum, Lc - Leptosynapta clarki, Mi - Molpadia intermedia, Pc - Parastichopus californicus. The solid triangle is the unusually late field spawning of P. chitonoides at Point George (see text).


Cps
Cl
Cm
Cp
Csp
Eq
Pp
Pch
Pb
Lc
Mi
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JAN
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JUNE
JULY
AUG
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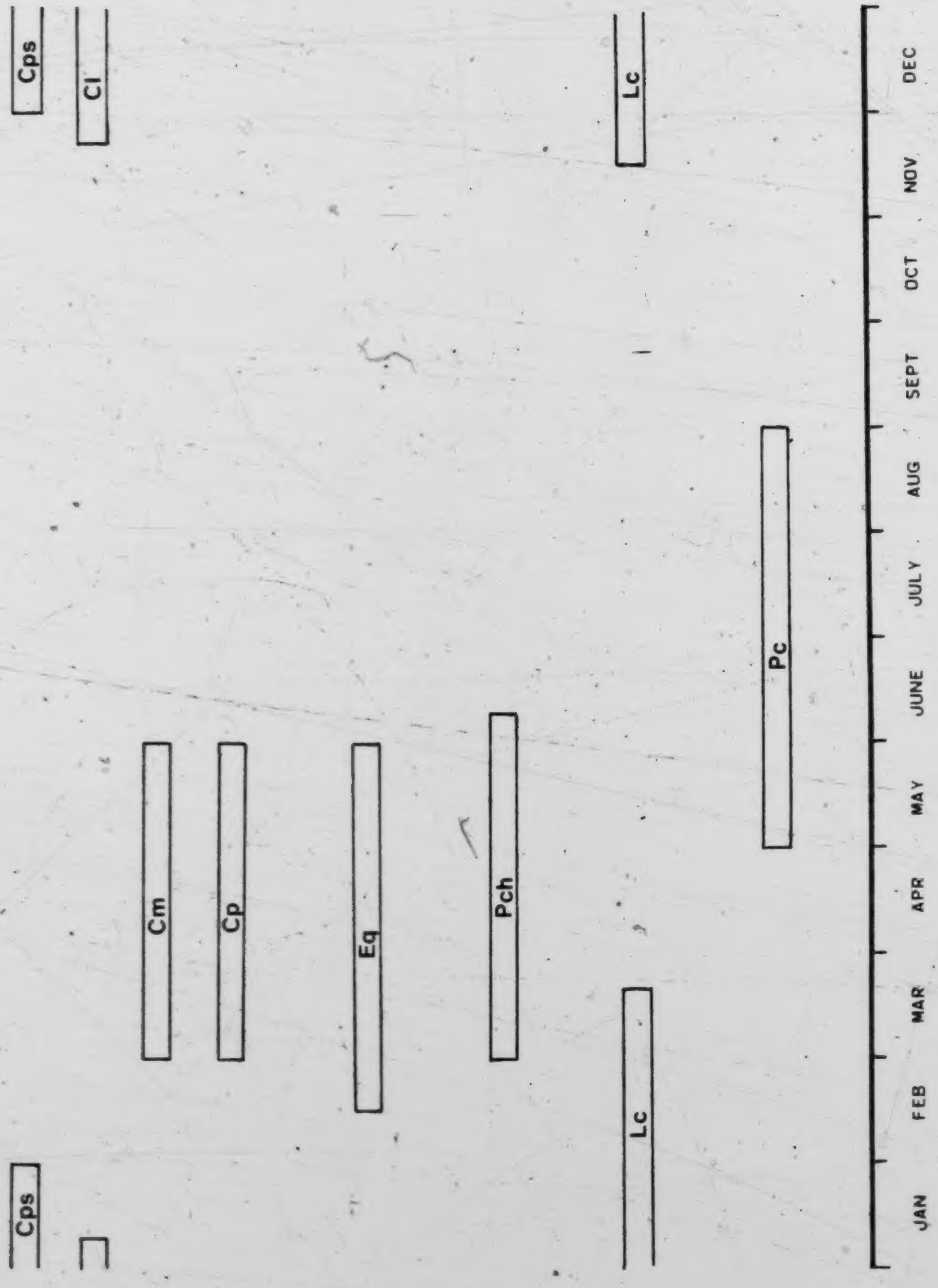
OCT
NOV
DEC

Fig. II-9. Estimated breeding seasons for holothuroids (eight species) based on published information (see text) prior to this study. Code same as for Fig. II-8.


Cps
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Lc
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JAN
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Psolus chitonoides

Johnson (1931) first noted that this psolid spawns in March; Johnson and Johnson (1950) found embryos in the plankton in March and June and reported spawning to occur in the spring months. Larvae in great numbers were collected by Jones (1960) in plankton tows from mid March into May, with larval numbers decreasing in the latter part of May and no larvae in the plankton in late June. She stated the breeding season to last from the middle of March into early June. Release of gametes in the laboratory has been recorded in March (Jones, 1960) and April (Young and Chia, 1982).

On 18 April 1983, all individuals sampled (25) at Point George were ripe, and on 5 May only one partially spent male was found among 22 ripe adults. Further sampling at Point George on 18 May revealed 24% of the adults (25 of 105) were spawned out, while at Shady Cove 64% (14 of 22 adults) had spawned by 27 May. The field spawning witnessed at Point George on 8 July, 1983, is considered unusually late and anomalous, because many large globules of degenerating oocytes (see chapter four) were present in the egg ropes. This same population was seen broadcasting gametes in mid April and late May 1981 (C. Young, personal communication). All individuals collected in mid June 1982 (n=41) had empty gonads.

Psolidium bullatum

Hadfield (1961) found little or no sexual activity during late fall and early winter and ripe individuals during late spring months. One partially spent male was found among 34 ripe adults collected at Point George the first week of April 1983. Two ripe males, four partially spawned out males, and 80 completely spent individuals were sampled from Bell Island and Point George on 9 June.

Cucumaria miniata

A spring spawning period was proposed by Johnson and Johnson (1950) who found several spent specimens in early May. Strathmann (1974) thought natural spawning to take place in late spring months, based upon her observations of spawning in tidepools in May and embryos in the plankton in April and May. For the population at Point George in 1983, all specimens were ripe in the second week of March (n=35), 62% (8 of 13) had spawned by 12 April, and no ripe gonads (in 18 adults) were found on 5 May.

Cucumaria piperata

Of 20 spent adults taken in East Sound by Johnson and Johnson (1950) in early July, there was one male "with a few active sperms". Sampling at Buck Bay (at mouth of East Sound) on 14 April 1983 showed this population had already spawned; all 104 individuals had empty gonads. By 20 April, 50% (6 of 12) were still ripe at Point George and 78% (22 of 29) had spawned by 30 April.

Cucumaria sp.

Inspection of 11 adults from Point George in the first week of March 1983 showed all of them to be ripe. Later collections on 20 April (n=20) and 22 May (n=26) had 50% and 77% spawned, respectively. Ninety three percent (14 of 15) Cucumaria sp. from Bell Island on 23 April were completely spent.

Eupentacta quinquesemita

Collected from the floats at the Friday Harbor town docks in the third week of March 1983, 43 adults of E. quinquesemita still possessed gonads full of gametes, by 28 May, 82% (n=101) were spent. Only spent females were found in early May by Johnson and Johnson (1950), who concluded this species spawns in spring. The largest gamete release on the west coast of Vancouver Island, as indicated from Byrne's (1983) monthly gonadal indexes, is from the middle of April into the last week of May, with some field spawning in February and March.

Pentamera populifera

In Cowlitz Bay, 1 of 119 P. populifera collected on 4 March 1983 had released its gametes. Ninety eight percent of the population had spawned out by 26 April (698 of 712).

Cucumaria lubrica

Atwood and Chia (1974) noted this species to brood in winter and collected mature males in December. Using laboratory and field data, Engstrom (1974, 1982) found spawning commenced in November and was complete by late December or early January. In 1981, brooding in the Point George population began between 2 December and 8 December. At Turn Island in 1982, broods were first produced between 17 November and 4 December; 2 of 100 adults inspected at Point George on 7 December of the same year had broods.

Cucumaria pseudocurata

Most reproductive information for C. pseudocurata comes from northern California. Brooding is known to occur in December at Pacific Grove (MacGinitie and MacGinitie, 1949); Smith (1962) found fertilized eggs and broods from late January to April. Brumbaugh (1964) stated C. pseudocurata spawns in late December and early January. Rutherford (1973, 1977) said eggs reach maturity in early December and are spawned in January and Turner and Rutherford (1976) found 1 - 2 day-old broods on 9 January. In the San Juan Islands, Atwood (1975a) collected mature males in December. My collections at Eagle Point and Grandmother's Cove on 10 and 12 December, 1981, respectively, revealed no brooding (n=75); brooding was discovered on 23 December at Grandmother's Cove, but not at Eagle Point. Laboratory-held specimens began releasing sperm on 22 December and by 8 January 1982, one third of the sample (14 of 42) inspected at Eagle Point had deposited eggs. The following breeding season (beginning December 1982), laboratory spawning commenced on almost the same day (21 December) as in the previous year; 3% (5 of 172) and 14% (5 of 36) of the the Eagle Point population were brooding on 20 and 22 December, respectively.

Parastichopus californicus

Examination of gonads from four different sites (Table II-3) supports findings of earlier workers for a May - July period of breeding in the San Juan Islands region. Bovard and Osterud (1918) obtained fertilization in July and ripe specimens were found in June on the east coast of Vancouver Island by Mortensen (1921). A late July - August spawning

Table II-3. Qualitative assessment of gonad condition of *Parastichopus californicus* from several sites in the San Juan Islands, Washington, May - July 1983. Number of large adults examined is in parentheses.

Site, date (# examined)	Sex	# unripe	# ripe	# partially spent	# spent	total % spent
Point George 18 May (26)	♂	1	11	0	1	31
	♀	1	5	0	7	
14 July (51)	♂	5	7	5	9	50
	♀	4	7	0	14	
Shady Cove 27 May (53)	♂	0	7	6	14	64
	♀	1	5	0	20	
East Sound 5 July (18)	♂	0	0	3	8	81
	♀	0	2	0	5	
29 July (31)	♂	0	0	2	15	97
	♀	0	0	0	14	
Bell Island 30 July (47)	♂	0	2	0	19	91
	♀	0	2	0	24	

season was proposed by Courtney (1927), but Johnson and Johnson (1950) suggested spring months when they were able to get successful fertilizations in the first two weeks of May. Occasional ripe individuals can be found in August (R. Miller, personal communication). Strathmann and Sato (1968) stated this species spawned in June, and Strathmann (1974) gave early summer as the spawning season, with ripe gametes being present in late April. Smiley (1984) outlined a period of spawning between the end of May and the middle of July and said gamete release is usually in late June in the San Juan Islands. In other locales, P. californicus has been seen spawning in situ in lower Puget Sound in the first week of July (R. Shimek, personal communication), while MacGinitie and MacGinitie (1949) report spawning in August at Newport Bay, California.

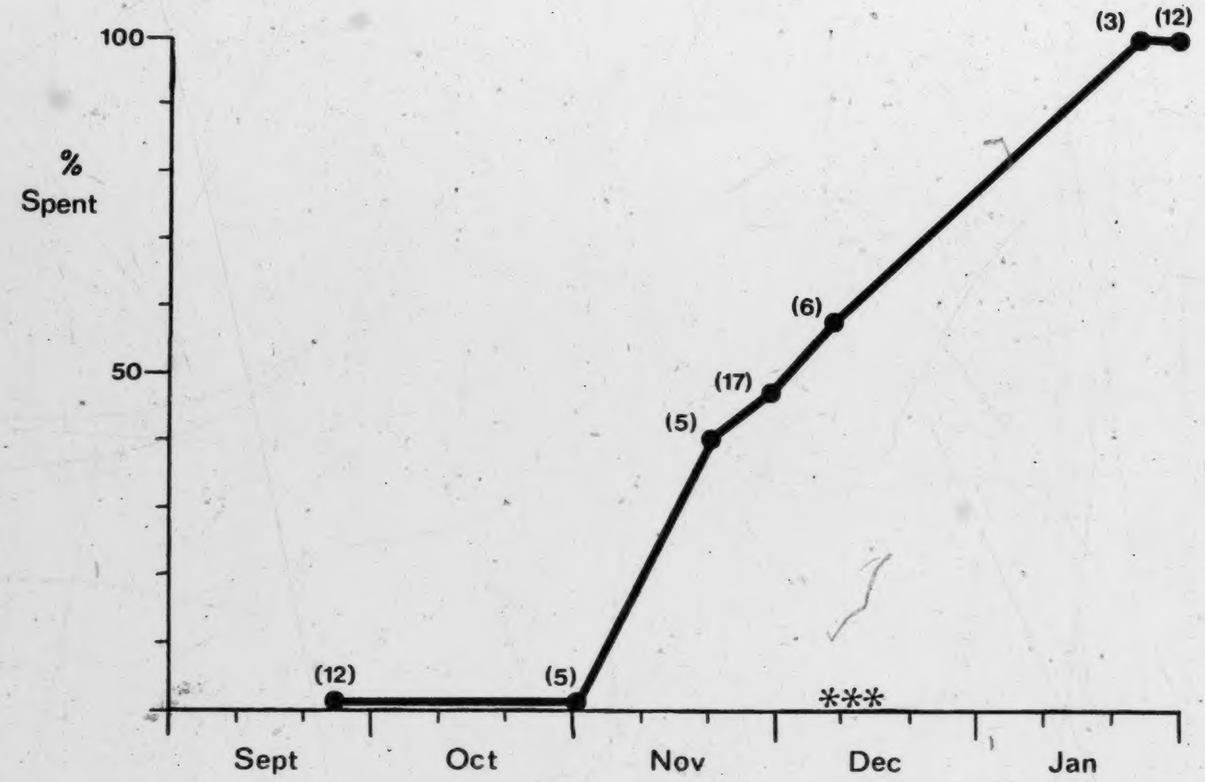
Leptosynapta clarki

In middle and late November, this ovarian brooding synaptid forms small breeding aggregations of 2 - 4 adults in False Bay (Everingham, 1961). I have come across these small groups of L. clarki as early as the last week of October and have also encountered blastula and gastrula stages in the ovaries in the second week of December. For the population at Sooke Reef, Vancouver Island, Brooks (1973) found ripe males December through April; she claimed fertilization took place between 16 February and 20 March after finding prepentactula embryos in March.

Molpadia intermedia

Although only small sample sizes were possible, inspection of gonads indicates a breeding period of November and December (Fig. II-10), which coincides with laboratory spawning. A more extended breeding season is probably the case because larvae have appeared in plankton tows taken during the last week of both September and October (plankton tows taken by M. Strathmann and R. Emlet).

Fig. II-10. Proportion of Molpadia intermedia with spent gonads in the period September 1982 through January 1983 (collected from East Sound, Orcas Island, Washington). Seven of the 12 adults collected on 24 September and 2 of 5 on 1 November contained unripe gonads. Dates with asterisks denote laboratory spawnings. Numbers in parentheses are sample sizes.


%
Spent
100
50
(12)
(5)
(5)
(17)
(6)
(3)
(12)
***
Sept
Oct
Nov
Dec
Jan

D. DISCUSSION

REPRODUCTIVE BEHAVIOR

A summary of reported laboratory and field observations of holothuroid spawning prior to this study is listed in Table II-4. Of the 43 species for which reproductive activity is known, almost half (18 species) belong to the order Aspidochirotida; this may be due to the fact that this holothuroid order is predominately tropical in distribution with many large species found in great numbers in easily accessible shallow bays, lagoons, and reef flats. Direct field observations of gamete release are, for the most part, relatively recent and restricted to one order. All accounts of field sightings date no earlier than 1963, with 7 of the 9 published reports of in situ behavior from 1979 and later. Only 16 species (and possibly one elasipodid species) have been sighted spawning in the field; 75% (12 species) of these are aspidochirotids.

A widespread behavioral trait exhibited by many representatives of most orders is a lifting or stretching of the anterior end of the body away from the substratum into the water prior to spawning (Table II-4). This type of behavior is especially well known for aspidochirotid holothuroids (Pawson, 1976) and has a correlate among asteroids and ophiuroids where the oral disc is elevated during the act of spawning and supported by the stiffened proximal, middle, or distal portion of the arms (ophiuroids: Heatwole and Stancyk, 1982; reviewed by Hendler and Meyer, 1982; asteroids: Galtsoff and Loosanoff, 1939; Christensen, in Feder and Christensen, 1966; Birkeland, 1970; Kanatani and Nagahama, 1983).

Frequently accompanying this upright posture is a back-and-forth swaying or swinging motion that has been noted in 7 species of aspidochirotids, 3 apodid species, and 1 dendrochirotid species (Table II-4); in my study this behavior was also observed in Parastichopus californicus, Cucumaria miniata, and Eupentacta quinquesemita. Undulatory motions in the latter two species took place in the laboratory when sea water circulation was cut off and when spawned gametes collected on the buccal membrane. Writhing or vigorous twisting can also be a part of the spawning display (Table II-4: Opheodesoma spectabilis, Parastichopus parvimensis, and Sclerodactyla (Thyone) briareus).

Table II-4. Review of direct observations of spawning and reproductive behavior of holothuroids. L: laboratory observations, F: field observations (habitat not specified), P: population, U: unspecified.

Species	# Individuals/ sex	Location	Time of day/ date	Behavior	References
Dendrochirotida					
Aslia lefevrei	U/both	L	evening, night, morning/Feb	♂ precede ♀; tentacles extended close together, anterior end raised; whole crown moves as single structure, tentacles wafted	Costelloe & Keegan, 1984; Costelloe (in press)
" "	U/both	L	night	---	Newth, 1916
Cucumaria echinata	U/both	L	evening/ June-Aug	♂ spawn before ♀	Ohshima, 1918
" "	6/♂, 2/♀	L	1315, 1700, 1930-2230 hrs/ June-Aug	♂ stretch out tentacles and keep them very quiet; ♀-no waving of tentacles; eggs released intermittently	Ohshima, 1921
C. elongata	U/both	L	early a.m./ Feb	wave back & forth; pharyngeal bulb and stationary tentacles fully extended; produce strings of eggs	Chia & Buchanan, 1969

C. frondosa	many/both	L	night	♂ spawn first	des Arts, 1910
" "	U/both	---	---	---	Runnstrom& Runnstrom, 1918-1919 (from Thorson, 1946)
" "	---	L(?)	Jan	---	Lonning, 1976
C. glacialis	U/♀	L(?)	---	eggs taken up (after being laid) from the sea bottom into brood pouches	Mortensen, 1894 (from Gerould, 1898)
C. lubrica	P/both	rock walls, boulders, L	♂: 1130-1400; ♀: 1630-1800/ Nov-Mar	♂ precede ♀; ♂ arch oral end away from substratum, tentacles crumpled; ♀ detach 2 ventral rows of tube feet, arch anterior end to deposit eggs between ventral surface and substratum	Engstrom, 1974, 1982
C. miniata	---	tide pools	May	during low tide	Strathmann, 1974
C. pseudocurata (C. curata)	U/♀	L	---	♀ arch expanded tentacles to catch extruded eggs; eggs transferred to ventral surface & arranged by podia	Smith, 1962
C. saxicola	U/both	L	night	---	Newth, 1916
Cucumaria sp.	U/♀	L	Apr	produce long strings of ova	McIntosh, 1889

Eupentacta quinquesemita	u/♀	L	Mar, Apr	eggs in compact strand	Strathmann, 1974
	---		Feb, Mar	---	Byrne, 1983
Lissothuria (Thyonepsolus) nutriens	U/both	L	2200 hrs/ all yr	♂ spawn first; ♀: arched tentacles form cuplike chamber that catches and holds eggs that are transferred to dorsal surface by 2 extensile pedicels and the tentacles	Wootton, 1949
Pentacucumis (Cucumaria) planci (=Pentacta doliolum)	---	L(?)	---	---	Kowalevsky, 1867 (from Ohshima, 1921)
" "	U/both	L	---	eggs transferred to atrium in front of mouth; spermatozoa thrown out by neighboring ♂ swept into atrium by tentacle movements of ♀; ♂ did not wave tentacles	Selenka, 1876 (from Gerould, 1898; MacBride, 1914; Ohshima, 1921)
Psolus chitonoides	U/both	L	0800, 1200, 1900 hrs/ Mar	cylindrical mucoid strand of eggs released; ♂ spawn first; ♀ tentacles widely extended	Jones, 1960
" "	P/both	cobble fields, rock walls, L	Apr	♂ initiate spawning; ♀ produce cylindrical egg strands	Young & Chia, 1982
Psolus phantapus	---	L	Feb, Mar	---	Thorson, 1946
Sclerodactyla (Thyone) briareus	many/ both	L	June, July	extended tentacles	Pearse, 1909
" "	---	L	late aft/ June	♂ spawn first	Ohshima, 1925

" "	many/U	L	---	----	Just, 1929
" "	35/♂, 14/♀	L	evening/ June	body expands to twice former length; wave tentacles in feeding movements; writhing movements precede shedding	Colwin, 1948
Stereoderma kirschbergi	U/both	L	--	♂ spawn first, sperm stirred up by waving tentacles, followed by ♀ 1 hr later	Kowalevsky, 1867 (from Ohshima, 1921)
Thyone fusus	1/U	L	---	--	Costelloe, pers. comm.
Aspidochirotida					
Actinopyga agassizi	---	shallow bay	dusk	stationary, raised posture	Hammond, 1982
A. serratidens	---	L	2000 hrs/ May	anterior end raised, but no swaying	Mortensen, 1937
Astichopus multifidus	---	shallow bay	dusk	stationary, raised posture	Hammond, 1982
Bohadschia (Holothuria) marmorata	U/both	L	evening, night/May	fore end raised almost vertically; raised fore ends move to side	Mortensen, 1937
" "	P/U	F	Dec	near identical behavior to H. scabra	Shelley, 1981
" "	many/ ♂(&♀?)	atop coral head	1420-1530, 2000 hrs/ July, Aug	anterior end lifted off substratum; tentacles shielded by tentacular collar; spawn on rising tides	Hendler & Meyer, 1982
Holothuria edulis	---	L	---	rise perpendicularly, standing on	Mortensen, 1938

				posterior tip of body; turn slowly around; may release hold and float freely at water surface	
H. *leucospilota*	P/U	1-2 m depth	1500 hrs/ June, July	2/3 of body in vertical position and waving	Toma, 1980
H. *mexicana*	3/♂	L	---	no rearing up nor waving; sperm ejected	Engstrom, 1980
" "	83/♂, 42/♀	shallow bay, L	late aft/ May, July-Sept	1/3-2/3 of oral end elevated perpendicularly, pursed mouth, undulating bows; ♀ weave and writhe, sometimes waving tentacles; gametes expelled with force; ♂ precede ♀	Mosher, 1982; pers. observ.
" "	---	shallow bay	dusk	stationary, raised posture	Hammond, 1982
H. (*Microthele*) *nobilis*	1/♂	lagoon grass flats, coral heads	Apr	one end elevated off substratum	Sloan et al., 1979
H. *poli*	---	L	---	---	Horstadius, 1925 (from Mortensen, 1938)
H. *scabra*	P/U	lagoon	1000, 1600-1630 hrs/ Nov, Dec	anterior end raised to vertical position and waves slowly around	Shelley, 1981
H. *spinifera*	"a number of specimens"	L	1800 hrs/ June	---	Mortensen, 1937
H. *thomasi*	---	dead coral	dusk	stationary, raised posture	Hammond, 1982

		heads			
H. tubulosa	---	L	---	♂ spawn first, emit sperm at intervals of 2-10 min	Selenka, 1876(from MacBride, 1914; Ohshima, 1921; Thorson, 1946)
Isostichopus badionotus	---	shallow bay	dusk	stationary, raised posture	Hammond, 1982
P. parvimensis	P/ both	5-11m depth, Cystoseira/ boulders	1600-1800 hrs/May, June	on highest portions of algae & boulders; in an "S" shape, stretched far out & attached by posterior tube feet, slowly waving free anterior end; some exhibit vigorous twisting	J. Engle, R. McPeak, pers. comm.
Stichopus tremulus					Selenka, 1876
" "	U/♂	L	---	anterior end raised above substratum	Nordgaard, 1912
" "					Runnstrom & Runnstrom, 1918-1919
" "	1/♂	L	after collection	---	Rustad, 1938
" "	several/U	L	---	---	Jespersen & Lutzen, 1971
" "	few/♂, 8/♀	L	morn, aft/ July	gametes squirted	Holland, 1981
Stichopus sp.	P/U	F	---	epidemic spawning	Dmitriev, 1955 (from Holland

					& Holland, 1969)
Thelenota ananas	P/U	F	---	similar to H. scabra	Halstead, in Shelley, 1981
aspidochirotid sp.	P/U	"Posidonie grass meadows"	noon/Apr	gather in pairs, body raised vertically & stretched out thin, sways back & forth; gametes jetted; fall back & rise 90 sec later	Cousteau & Dugan, 1963
many species (assumed to be aspidochirotids)	---	F	---	shoreward migrations during breeding time	Deichmann(in Glynn, 1965
Elasipodida					
Orphnurgus (or Pannychia)	2/U	F	---	front half of body raised off substratum(no gametes seen)	Pawson, 1976
Apodida					
Labidoplax buskii	---	L	no particular time/ Oct-Dec	(hermaphroditic) eject one kind of sexual cell at each spawning; lean forward & sway backwards & forwards a half or whole turn; eggs ejected in mucus	Nyholm, 1951
Leptosynapta clarki	---	sandflats	Nov	2-4 animals lie in sand close together or intertwined(no spawning seen)	Everingham, 1961
L. inhaerens	76/♂,4/♀	L	1500-1800 hrs/June, July	♂ spawn first; erect themselves part way out of burrows & swing about while emitting sex cells	Runnstrom, 1924, 1927(from Thorson, 1946; Hyman, 1955; Costello et al., 1957)

Opheodesoma spectabilis	P/U	*Sargassum* bed	evening/ Sept	erect anterior part of body free from substratum, sway and twist while tentacle rhythm continues	Berrill, 1966
Molpadiida					
Caudina arenata	several/ ♂	L	Feb, Mar	---	Gerould, 1896
Paracaudina (Caudina) chilensis	9/both	L	evening/ May, June	spawn during high tide; body and tentacles of both sexes kept entirely quiet	Inaba, 1930

Tentacle waving is another of the more obvious behaviors among spawning sea cucumbers and appears to be most common among dendrochirotids. Previous workers have related how tentacle movements in male dendrochirotids stir up sperm (Kowalevsky, 1867; Colwin, 1948) and, in females, aid fertilization by sweeping sperm into brood cavities where eggs are held (Selenka, 1876). My observations on six dendrochirotid species (Cucumaria miniata, C. piperata, Cucumaria sp., Eupentacta quinquesemita, Pentamera populifera, and Psolus chitonoides) also verify that there is direct contact of waving tentacles with newly released sperm and eggs, often with the tentacles swabbing the papilla and then extending out to release the gametes. Waving of tentacles also occurs in Opheodesoma spectabilis (Berrill, 1966), Leptosynapta clarki (this study), and sometimes in Holothuria mexicana (Mosher, 1982), but the extent to which the tentacles interact with gametes for these three species is not yet clear. Similar behavior for spreading sexual products is exhibited by other groups of echinoderms: ophiuroids and crinoids either undulate or thrash their arms (Hendler and Meyer, 1982 and references therein) and echinoids move their spines (Chia, 1977; Pawson and Miller, 1979). As well, whipping movements of tube feet in ophiuroids (Woodley, 1975; Hendler, 1977) and movements of both tube feet and pedicellariae in echinoids (Pawson and Miller, 1979) help in dispersing gametes. Similar tube foot activity among holothuroids is known only for C. miniata and C. piperata (this study).

An exceptional method employed by some sea cucumbers to ensure that gametes are suspended in the water column is forceful expulsion. This is especially pronounced in females of Molpadia intermedia (this study) and Holothuria mexicana (Mosher, 1982; personal observations) that either spew out all the eggs in one burst or in a number of successive emissions, respectively, in contrast to slow dribbling or thin streams of eggs and sperm. In similar fashion, the gametes of Stichopus tremulus are "squirted" (Holland, 1981) and, for an unidentified aspidochirotid species, Cousteau and Dugan (1963) say the gametes are "jetted".

The mechanism by which the above-mentioned species expel their gametes with force is not known, but the probable sequence of steps involved for female Molpadia intermedia is outlined below, based upon the reproductive behavior reported here and upon morphological studies of earlier investigators.

1. Eggs are pushed out of the ovarian tubules by contractions of muscles in the ovarian wall (reviewed by Kanatani and Nagahama, 1983; Chia and Bickell, 1983).
2. Eggs extruded from the ovaries are collected in the gonoduct which is expanded greatly by the accumulation of gametes. The gonoduct of female molpadiids is more voluminous with thicker walls than that of males (Heding, 1935; this study), most likely due to a greater musculature and overall mass to accommodate a large volume of eggs. Gerould (1896) described circular muscle fibers forming a sphincter in the connective tissue of the anterior terminal part of the gonoduct in Caudina arenata; he also found that the connective tissue at the tip of the genital papilla forms a thin layer continuous with the body wall. Tissue blockages of the genital pore are also known for the molpadiids M. roretzii (Hatanaka, 1939) and M. intermedia, as well as for dendrochirotids (this study). The sphincter muscle and blocked gonoduct are thought to act together to contain the eggs within the duct.
3. The gonopore sphincter muscle relaxes and the longitudinal muscles of the gonoduct (Danielssen and Koren, 1882; Gerould, 1896) contract, forcing the eggs upward and ripping the connective tissue blocking the end of the gonoduct that results in a pressurized discharge. Circular muscles in the gonadal basis (Gerould, 1896) at the posterior end of the gonoduct presumably contract before or simultaneously with the gonoduct muscles and prevent eggs from returning into the ovarian tubules.

Probably the most elaborate form of reproductive behavior among free-spawning echinoderms is "pseudocopulation", a kind of copulation known for three asteroid species (Boschma, 1923; Mortensen, 1931; Ohshima and Ikeda, 1934; Clemente and Anicete, 1949; Kubo, 1951; Janssen et al., 1984; McEuen, unpublished data) where the male is positioned on the aboral side of the female with arms interlocking with those of the female. The only similar behavior known for ophiuroids comes from Mortensen's (1933, 1936) and Gorzula's (1974) reports of dwarf males that ride permanently on the oral or aboral sides of the females. At spawning time, the echinoid Psammechinus miliaris associates in pairs, most of which consist of opposite sexes (Orton, 1914). An observation of paired Parastichopus californicus adults wrapped around each other while spawning (J. Elliott, personal communication) might also be considered a form of pseudocopulation; another aspidochirotid species has also been reported to free-spawn after gathering in pairs (Cousteau and Dugan, 1963). Aggregations also appear during the reproductive season in Leptosynapta clarki; the adults intertwined in small groups of 2 - 4 individuals during October and November (Everingham, 1961; this study).

Although aggregations are not uncommon among echinoderms (Reese, 1966; Warner, 1979; Sloan, 1980), the above associations are the only instances known for which holothuroid aggregations occur specifically for reproductive purposes. Deichmann (in Glynn, 1965) stated that tropical holothuroids (probably referring to aspidochirotids) move into shallow water for reproduction, but no specific observations nor other data were provided. Higher percentages of mature P. californicus in shallow water than in deep water caused Courtney (1927) and Eschenberg (1952) to speculate that spawning is chiefly in shallow water, implying migration from deeper water. Thorson (1946) emphasized the importance of high density populations of many benthic marine invertebrates as advantageous for increased chances of fertilization, but the occurrence of dense beds of sea cucumbers have thus far been interpreted mostly as a recruitment phenomenon or as migrations of juveniles and adults to better feeding grounds (Konnecker and Keegan, 1973; Billett and Hansen, 1982; Chia et al., 1984; appendix one).

GAMETE PROPERTIES

Free-spawning echinoderms have eggs that are slightly negative, neutral, or positively buoyant; to my knowledge, no echinoderms broadcast heavy eggs. It might be proposed that, since slightly negatively and neutrally buoyant echinoderm eggs released into the plankton are less than 200 um (though there are some as large as 400 um; chapter five), suspension in the water column would be no problem due to the eggs' relatively large surface area-to-volume ratio and their neutral (or nearly neutral) buoyancy. However, field observations show that not all eggs are water-borne right after spawning. For Pentamera populifera and the asteroid Pycnopodia helianthoides, large quantities of newly shed eggs were witnessed to fall onto the substratum in the course of field spawnings. Fishelson (1968) also saw that a large number of eggs from a free-spawning crinoid in the

Red Sea fell into a crevice. If these eggs are not subsequently stirred up and dispersed by tidal currents, high mortality would then be assumed to follow as a result of predation, siltation, microbial infection, or failure of fertilization. In the event that these temporarily benthic eggs are fertilized, hatching (with the resultant free-swimming larva) would function to free the early embryos from benthic hazards and would suspend them for dispersal. At temperatures of 5 - 30° C, hatching times for neutrally or slightly negatively buoyant eggs from broadcasting echinoderms typically range from 5 - 48 hrs from fertilization. The fate of eggs in this interval from the time of release from the gonopore to hatching is obviously influential in determining the size of that year's larval cohort.

The floating eggs of Cucumaria miniata, C. piperata, Cucumaria sp., and Psolus chitonoides are packaged in pellets, strands, or ropes that float to the water surface and there break apart. One consequence of compacting floating gametes into bundles is that the rate of rising of an egg pellet is much greater than that of a single egg (F. S. McEuen, unpublished data) because of the pellet's smaller surface area-to-volume ratio. Several potential fertilization problems confront packaged eggs. Although eggs and sperm are released synchronously, pellets and ropes may ascend too rapidly through the cloud of sperm produced by neighboring males, resulting in reduced fertilization rates. Of possibly greater significance would be the failure of spermatozoa to reach eggs inside the pellets and ropes. It is doubtful that the rounded, primitive-type sperm of these sea cucumbers (Chia et al., 1975; chapter five) could penetrate the mucous substance binding the pellets and ropes together. The viable life span for newly shed, diluted eggs and sperm is not known for holothuroids, but may be critical in its influence on fertilization rates for sea cucumbers with buoyant eggs.

Both Cucumaria lubrica and C. pseudocurata appear to increase their fertilization rates by enmeshing spermatozoa in bundles and thick streams of mucus (Figs. II-4D-G, II-5C) that are resistant to immediate dilution. Delaying the respiratory dilution effect (Chia and Bickell, 1983) in this manner may be essentially a way of transporting "dry" sperm to the female's and insuring viable sperm are present when eggs are laid. Assuming the spawning peaks of C. lubrica males and females in Figure II-7 reflect field behavior, most females begin releasing eggs 3 - 4 hrs after males commence spawning. This and Engstrom's (1974, 1982) data on delay of egg-laying in C. lubrica emphasize the need for morphological or behavioral aids to insure gamete union. Successful fertilization in this species is further enhanced by multiple spawnings of individual males and the males' errant behavior. Fertilization in the brooding *Lissothuria (Thyonepsolus) nutriens* is also thought to be facilitated by male nomadism (Wootton, 1949).

The "waste" of marine invertebrate eggs due to failure of fertilization at the time of spawning was considered by Thorson (1946) to be extremely slight because of adult aggregations and epidemic spawning. However, no data on fertilization rates have yet been gathered from natural spawnings of invertebrates *in situ*. From laboratory and field experiments where echinoids were induced to spawn, Pennington (1984) showed that zygote production could be substantially reduced due to gamete dilution if females were not close to spawning males. The July spawning of *Psolus chitonoides* at Point George bespeaks another source of decreased zygote production: oocyte degeneration as a result of postponed gamete release. How widespread these situations are that produce low numbers of embryos is not known, but do suggest that populations in favorable circumstances for gamete production and fertilization (high density, abundant food supply, suitable current regime, synchronized gametogenesis and spawning) may supply a high proportion of the larval recruits to neighboring populations.

The recent studies on echinoderm sperm chemotaxis by Miller (1985) present particularly interesting problems with regard to fertilization in holothuroids. Using 9 of the 12 species employed in this study (all but *Cucumaria lubrica*, *C. pseudocurata*, and *Leptosynapta clarki*), he demonstrated much cross-reactivity among sperm and egg extracts of the dendrochirotid sea cucumbers. Miller's data indicate that heterospermy may occur among sympatric species with overlapping breeding seasons. This would appear applicable to *C. miniata*, *Eupentacta quinquesemita*, and *Psolus chitonoides*, which live in close association in cobble and boulder fields and on rock walls. These dendrochirotids may avoid interspecific gamete mixing with staggered spawning through the day and night. The laboratory evidence gathered here (Figs. II-6, 7), as well as the field spawning data, suggest the following hours of spawning: *P. chitonoides*, early morning daylight hours; *C. miniata*, late morning and afternoon; *E. quinquesemita*, evening and early hours of the night.

Although the above three species were never seen releasing gametes together in the field, *Cucumaria miniata* was witnessed spawning *in situ* on one occasion at two different sites simultaneously with *C. piperata* and *Cucumaria* sp. Heterospermy, then, is probably common in some locales for these three cucumariids. The habitats where the largest populations of these species are found, however, are quite distinct: *Cucumaria* sp. is abundant in cobble fields in the quiet waters of inlets and fjords (P. Lambert, personal communication; personal observation), *C. piperata* occurs in vast beds on mud bottoms, and *C. miniata* dominates cobble fields in channels and passes swept by tidal currents. Therefore, if contamination by interspecific gametes decreases fertilization rates, spatial separation of the major populations of the three species would serve to prevent this problem, despite gamete mixing at some localities.

SECONDARY SEXUAL CHARACTERISTICS

To date, very few external morphological characters have been found for holothuroids that are reliable indicators of sex. The branching of the male papilla is sometimes useful in separating males and females of larger dendrochirotids (*Cucumaria* sp., *C. miniata*, *C. piperata*, *Psolus chitonoides*). Multlple gonopores in male dendrochirotids (Table II-1) are interpreted as an aid promoting sperm dispersal. In other cucumariids, there are also fewer gonopores among females while male gonopores may number more than 50 (Edwards, 1910 a, b; Ohshima, 1921). Conversely, reduction of gonopore number and the elongate papilla of male *C. lubrica* and especially *C. pseudocurata* help form the sperm strands or strings that restrict sperm dilution. Dimorphic genital papillae are also present on some sea urchins. The longer, tubular papillae of male echinoids are thought to facilitate sperm dispersal, but why there is such an irregular distribution of dimorphic genital papillae among echinoid species is still uncertain (Chia, 1977; Pawson and Miller, 1983).

SPAWNING STIMULUS AND HOUR OF SPAWNING

Environmental cues that induce spawning have been suggested for many marine invertebrates, but few in-depth studies have isolated the specific stimuli for spawning (see Introduction in Giese and Pearse, 1974). For holothuroids, only a handful of workers have proposed factors that might be responsible for regulating reproductive behavior, partially as a result of the fact that echinoids are such suitable subjects for experimentation and that holothuroids have traditionally been regarded as difficult to collect and have yielded poor results when attempts at artificial fertilization have been made (Selenka, 1876; Brooks, 1888; Edwards, 1889, 1909; Clark, 1898; Mitsukuri, 1903; Mortensen, 1913, 1921; MacBride, 1914; Newth, 1916; Ohshima, 1921; Nyholm, 1951; Inaba, 1968; Maruyama, 1980).

In the past, a number of workers (Edwards, 1889, 1909; Inaba, 1930; Mortensen, 1937) have been able to collect early embryos and larvae of sea cucumbers by employing Selenka's "live-box" method (Selenka, 1876). Holothuroid gametes for culture have sometimes been obtained from spawnings induced by stresses from handling during collection (Mortensen, 1938; Rustad, 1938; Engstrom, 1980; Holland, 1981), but few holothuroids have thus far responded in a predictable manner to efforts of investigators to elicit gamete release using artificial methods. Colwin (1948) gave some evidence that warming adult *Sclerodactyla (Thyone) briareus* for more than 3 hrs and cooling afterwards would produce spawning. Ohshima (1925) caused spawning in the same species by placing individuals in dim light. Spawning is readily evoked in *Holothuria mexicana* when ripe adults are placed in containers and "covered for darkness" (Mosher, 1982; personal observations). Injections of solutions of KCl and NaCl resulted only in waving tentacle movements (caused by NaCl) in *S. (Thyone) briareus* (Pearse, 1909). KCl injections and gonad-stripping for artificial fertilization both failed for *Cucumaria frondosa* (Jordan, 1972), but females released eggs after the seawater temperature was dropped by 2° C and then warmed in a dense concentration of *Dunaliella* sp. This latter method has also proved effective with *S. (Thyone) briareus* (Colwin and Summers, in Jordan, 1972). Horstadius (1925) increased pH to obtain gametes from *Holothuria Poli*, but subsequent attempts with this procedure have proven unsuccessful (Mortensen, 1937, 1938). Mortensen (1937), on some occasions, was successful in inducing shedding by making a slit in the body wall. With *Cucumaria echinata*, Mitsukuri (in Ohshima, 1921) claimed that it was "necessary to tease the animal and not change the water." Some of the stimulants used in bringing about spawning under laboratory conditions may indeed be the very same that trigger field spawning, but the large variety of methods and chemical compounds known to cause gonadal muscle contractions in marine invertebrates prevents definite conclusions on what the natural cues are when based solely on laboratory results.

Although laboratory conditions may hasten or delay reproductive activities, natural spawnings in the laboratory (where no deliberate measures are taken to induce) can serve as useful indicators of likely exogenous factors prompting field spawning. Some laboratory-held echinoderms have been noted to shed gametes at specific times of the day (Mortensen, 1937, 1938; Dan and Dan, 1941; Kume and Dan, 1968). Hyman's (1955) generalization that most holothuroids spawn in late afternoon, evening, or during the night was probably in reference to aspidochirotids, since 18 of the 27 sea cucumber species known to spawn in the field or laboratory during these late hours are aspidochirotids (Table II-4; Mortensen, 1937, 1938). Seventeen of these 18 aspidochirotid species have tropical distributions. Two exceptions for time of spawning among these aspidochirotids are: 1) isolated individuals of Holothuria scabra spawning at 1000 hrs (Shelley, 1981) and 2) a few Stichopus tremulus spawning in the morning after collection (Holland, 1981). The evidence to date, then, shows that heavy spawning in aspidochirotid populations takes place in the afternoon into the late evening and night and may relate to a decrease in light intensity.

Light was indicated as a potential spawning trigger for Leptopentacta (Cucumaria) elongata (Chia and Buchanan, 1969) and may well serve as a cue for the psolids Psolus chitonoides and Psolidium bullatum. The facility with which gamete release is brought on in P. chitonoides when ripe individuals are exposed to higher light levels, the frequent morning spawnings in laboratory tanks (Fig. II-6) on sunny days, and a field sighting of the population at Point George free-spawning at 0930 - 1030 hrs all seem to point to an increase in light intensity as the spawning stimulus for these two psolids.

Cucumaria miniata released gametes naturally in seawater tables throughout all daylight hours (Fig. II-6), but proved quite refractory to artificial techniques. Mass field spawnings in four separate populations also occurred in daylight (0930 - 1400 hrs) and in the course of a dense phytoplankton bloom (visibility in the green water was reduced to 3 - 4 m) during the last two weeks of March and first week of April, 1983. Himmelman (1975) demonstrated that phytoplankton is a major cue for gamete release for nearby populations of an echinoid and two chitons on the coast of Vancouver Island, and phytoplankton as well may initiate spawning in C. miniata and the four other echinoderms (C. piperata, Cucumaria sp., Pycnopodia helianthoides, and Solaster dawsoni) observed spawning in the field concurrently with C. miniata during heavy phytoplankton production. The release of gametes from ripe C. frondosa in response to increased algal concentrations (Jordan, 1972) lends additional support to the idea of phytoplankton as a proximate cue. Cucumaria frondosa lives in cold temperate waters on the east of North America and is very similar to C. miniata in size, abundance, habitat, and breeding season (Edwards, 1910a; Runnstrom and Runnstrom, 1918 - 1919; Thorson, 1946; Jordan, 1972).

Despite the emphasis of many investigators on the various factors that are thought to synchronize, induce or enhance gamete release among free-spawning marine invertebrates, little consideration has been given to the potential effects of current speeds and tidal patterns upon fertilization success. Dendrochirotids dwelling in rocky habitats in the narrow channels and passes that separate the islands in the San Juan Archipelago are subject to swift tidal currents that flush the greater Puget Sound region twice a day. San Juan Channel (and probably the Wasp Passage/Pole Pass area), experiences bottom current velocities of 1.5 - 2.5 knots, with 2 - 4 knot currents in the scour channel close to Point George (Wennekens, 1959). Powerful tide rips near prominent points and banks sometimes reach velocities of 7 knots (Thompson and Robinson, 1934). In these areas, the large populations of dendrochirotids in cobble fields and on boulders and rock walls broadcast their gametes during the periods of slack water, when conditions would seemingly be better for gamete mixing and fertilization, thereby avoiding too rapid dilution and loss of gametes by strong currents. The diving observations reported here were necessarily made during intervals of weak current, but the likelihood of spawning taking place under stiff current conditions is thought to be small since strong currents press the tentacles flat against the substratum and cause suspension-feeding sea cucumbers to completely withdraw (Konnecker and Keegan, 1973; personal field observations). Spawning was also enhanced in the laboratory by turning off seawater circulation. Some of the numerous instances of laboratory spawning of marine invertebrates reported to have been triggered by an increase in temperature may have actually been due to calm water conditions in the holding vessels, especially if the adults live in habitats that regularly experience high current velocities.

As a contrast, holothuroids living in shallow bays and mud bottoms seem to respond with reproductive behaviors appropriate to low-current conditions for increased fertilization success. More individuals of Pentamera populifera spawn when a slow steady current is flowing than in still water. This may be comparable to current speeds present when rock-dwelling dendrochirotids spawn during slack water because, owing to the uneven bottom topography, upwellings, cross-currents, and the large exchange of water volume between high and low tides, completely still water is never encountered in rocky habitats in the high current channels among the San Juan Islands. The four holothuroids mentioned above that spew out their gametes under pressure have another shared feature in that all are residents of low-current habitats: M. intermedia burrows in fine mud in East Sound where current velocities are less than 0.5 knots (Wennekens, 1959), H. mexicana is found at shallow depths in Caribbean lagoons and enclosed bays (Hammond, 1981; personal observations), S. tremulus is considered a "mud-dweller" in Swedish fjords (Rustad, 1938; Jespersen and Lutzen, 1971; Holland, 1981), and the aspidochirotid described by Cousteau and Dugan (1963) lives in Mediterranean seagrass meadows. This forceful release of gametes is interpreted as an effort to suspend eggs and sperm in the water column in consequence of current speeds being less than adequate for gamete mixing.

My field and laboratory observations of cucumber swaying and tentacle waving took place under low current conditions when gametes fell onto the buccal membrane. Crinoids as well display variable spawning behavior in the field: a Red Sea crinoid (Lamprometra klunzingeri) vigorously lashed its arms while most of the eggs fell into a crevice (Fishelson, 1968), but Hendler and Meyer (1982) photographed and described the stationary, extended arm posture of Capillaster multiradiatus in the Palau Islands. Eggs of the latter were being lifted up and away by currents. Low current speeds and the presence of spawned gametes on the body wall very likely induce modified behavior in broadcast spawning echinoderms. It is hypothesized that for free-spawning benthic marine invertebrates, slow current velocities are optimal for fertilization, and where current conditions are adverse, selection for modification of behaviors, morphology, and gamete properties will promote the likelihood of gamete union. Time of year, time of day, spawning synchrony, and spawning behavior all coordinate to maximize rates of fertilization in the particular habitats of the species.

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III. LARVAL DEVELOPMENT OF A MOLPADIID HOLOTHUROID, MOLPADIA INTERMEDIA (LUDWIG, 1894) (ECHINODERMATA)[1]

A. INTRODUCTION

Sea cucumbers in the order Molpadiida are commonly found in mud and sand bottoms of world seas to depths of 6 000 m (Clark 1907, 1920; Madsen 1947-1948; Pawson 1977). These sausage-shaped deposit feeders can reach densities of 147/m^2 (Knox and Fenwick 1981) and are important in mud communities in reworking and oxidizing large quantities of sediment and in producing spatial habitat diversity for surface-dwelling suspension feeders (Tao 1930; Rhoads and Young 1971). Of the approximately 85 species in the order Molpadiida, 55 belong to the family Molpadiidae and most of these have deposits of iron phosphate in their body wall and are cosmopolitan in distribution (Morner 1902; Clark 1907; Pawson 1963, 1970, 1982; Pawson *et al.* 1975).

Despite their ecological importance in mud communities, little is known about the reproduction of this group of echinoderms (Hyman 1955; Boolootian 1966; Pawson 1977). There is but one study on the development of Molpadiida, by Inaba (1930), who described the embryology of a caudinid, Paracaudina (Caudina) chilensis. Electron microscopic examinations of sea cucumber reproduction are limited to studies of the development of egg through hatched blastula of Stichopus tremulus (Holland 1981) and of sperm-egg interactions (Colwin *et al.* 1975). In the present study, scanning electron microscopy is used to provide a detailed account of the larval development of Molpadia intermedia, a dark grayish-purple molpadiid sea cucumber which, according to Clark (1907), is "the common Molpadia of the eastern Pacific." In the northeast Pacific, this species is considered a dominant member of subtidal mud bottoms and contributes up to 63% of macrofaunal biomass with densities of 15/m^2 (Ellis 1969; Nichols 1975; Levings *et al.* 1983).

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[1] A version of this chapter has been accepted for publication. McEuen, F. Scott and Fu-Shiang Chia: Larval development of a molpadiid holothuroid, Molpadia intermedia (Ludwig, 1894) (Echinodermata). Can. J. Zool. *63* (1985).

B. MATERIALS AND METHODS

Large adults of Molpadia intermedia were collected at East Sound and Buck Bay (Orcas Island) (48° 38' N. and 122° 51' W.) and at Cowlitz Bay (Waldron Island) (48° 42' N. and 123° 2' W.), Washington, using SCUBA and occasionally by dredging. Most collections were made during dives in East Sound at depths of 26 - 28 m where adults measuring 35 - 43 cm (relaxed feeding state) were found buried as deep as 35 cm in very fine soft mud. Individuals tended to be aggregated in groups of approximately 2 - 6 in an area of about 1 - 2 m^2. Collected specimens contracted to lengths of 15 - 18 cm and a maximum width of 6 cm. The depth to which M. intermedia burrows into mud appears to be regulated by the degree of compaction of the sediment. In firm mud at 15 - 21 m in Buck Bay, M. intermedia was encountered only 5 - 8 cm beneath the surface. This sea cucumber was also present 10 - 20 cm below the mud surface at shallow depths of 7 - 9 m in Cowlitz Bay.

Adult individuals were maintained at Friday Harbor Laboratories in 5-gallon plastic aquaria with a 10 - 20 cm layer of soft mud at the bottom. A slow but constant flow of seawater was maintained in these containers. In this manner, individuals were successfully kept for 2 1/2 - 4 months with a minimum of care.

Embryos and larvae for culture were obtained through artificial fertilization of gametes taken from ripe adults and of gametes spawned naturally in the laboratory. Eggs were selected from the lumen of the ovarian tubules of ripe females. For artificial fertilization, 0.5 ml of dilute sperm suspension (one drop "dry" sperm from spawning males or from ripe testes, diluted with 50 ml of 0.45 um mesh size Millipore-filtered seawater) was added to every 50 ml of seawater with 50 - 75 eggs; after 10 min, eggs were rinsed several times with filtered seawater. Fertilized eggs (150 - 250) were added to each culture bowl containing 300 ml of filtered seawater and maintained in an incubator at 11° ± 0.5°C, with a 12-hr photoperiod using flourescent light for uniform temperature and light conditions. Five bowls of the incubated embryos from three females were monitored for development schedule. Timing of developmental stages was determined when 50% of the incubated embryos or larvae had reached a particular stage. Additional cultures were also kept in seawater tables.

Embryos and larvae for scanning electron microscopy (SEM) were kept clean with daily changes of Millipore-filtered seawater during early embryonic development and every 2 - 3 d during the later, swimming, larval stages. For fixation, embryos were pipetted into small glass vials containing a few ml of Millipore-filtered seawater. 10 - 15 drops of 4% osmium tetroxide were added. After 15 - 20 min, the material was rinsed briefly with Millipore-filtered seawater, rinsed 15 min in distilled water, and serially dehydrated up to absolute ethanol using 15 min changes of 25, 50, 70 and 85% ethanol. Specimens were transferred gently into amyl acetate by adding amyl acetate dropwise every 5 min over a period of 30 min, beginning with one drop and then doubling the amount at each 5 min interval. This was followed with 0.5 ml additions of amyl acetate every 15 min. Larval stages and pentactulae with retractile tentacles were pipetted live into small individual beads of Millipore-filtered seawater on a hydrophobic surface (plastic weigh boat) and allowed to relax. A few drops of 4% osmium were pipetted in rapid succession directly onto each bead of water, fixing the larvae and pentactulae instantly in natural positions with a minimum of shrinkage and distortion. After a brief rinse in Millipore-filtered seawater, material was transferred to glass vials, with subsequent procedures the same as those described above for embryos. Amyl acetate proved to be a superior dehydrating agent to other solvents used (e. g., 2,2-Dimethoxypropane). Dehydrated specimens were then critical point dried, mounted on aluminum studs, sputter coated with 200 Å of gold, and viewed with SEM. When specimens were stored in 70% ethanol, cracking of the epidermis of embryos and later stages and excessive loss of larval cilia were avoided by completing the dehydration process and SEM examination within 2 - 3 wk of fixation.

C. RESULTS

EMBRYONIC AND LARVAL DEVELOPMENT

The long, slender, orange ovarian tubules of Molpadia intermedia become pinkish orange when ripe. In the ovary, the ripe oocyte has a conspicuous "micropyle process" and is surrounded by a jelly coat with follicle cells (Fig. III-1A); the latter is shed before spawning. Primary oocytes, obtained from the ovaries in September, possess a discrete ring of orange droplets at the animal pole (Fig. III-1B); this ring loses its ring-like character and becomes an orangish-pink cap by the time of spawning (Fig. III-1C). The orangish-pink droplets concentrated in the apical end are semi-opaque and evenly pigmented; they are assumed to be yolk as eggs and developmental stages always orient with the apical end up, similar to larvae of other holothuroids with a yolky pre-oral lobe (chapters three and four). The ripe egg is light orangish-pink and negatively buoyant, and averages 267 ± 3 um (s.e.) in diameter (n=20, 10 eggs each from two females). The sperm head is spherical and is considered to be primitive type sperm.

The onset of spawning is accompanied by unusual behavior. In the early part of December in the laboratory, males rose vertically with the anterior end above the mud bottom and released intermittent puffs of sperm; one female also rose above the substratum and spewed out all the eggs in one powerful explosive burst.

Spawned eggs were collected and placed in culture dishes where they rested on the bottom of the dish with the yolky end facing upward. Fertilization success with spawned eggs was 99.9% while only 0.5 - 1.0 % fertilization was obtained with eggs extracted from the lumen of ovaries in September. The second polar body is extruded from the area of the yolk cap in an eccentric position, sometimes close to the yolk cap area, by 1.5 hr following fertilization (Fig. III-1C). First cleavage is completed at 2.25 hr after fertilization (Fig. III-1D). Successive cell divisions are regularly spaced at 75-min intervals through the 32-cell stage (Fig. III-1E, Table III-1). The first two cleavages are meridional and the third cleavage is equatorial with ensuing cleavages alternating between meridional and equatorial. Cleavage is equal and holoblastic, although a few embryos were seen with slightly subequal cell divisions.

Fig. III-1. Molpadia intermedia. (A) Oocyte (taken from ovary) with coat of follicle cells micropyle process not visible in this micrograph. (B) Oocyte with discrete ring of yolk encircling the animal pole. (C) Recently spawned telolecithal egg with cap of yolk and extruded polar bodies (latter are to left of cap of yolk). Scale bar=75 um. (D) 2-cell stage showing equal holoblastic cleavage (2.25 hr post-fertilization). (E) Apical view of second cleavage. (F) 64-cell stage. (G) Early coeloblastula. (H) Hatching blastula emerging from egg membrane. (I) Proliferation of mesenchyme cells from vegetal pole in the late blastula. (J) Invagination in early gastrula. (K) Coelomic pouches (p) on either side of the archenteron in the early doliolaria larva; arrows indicate epidermal ridges of future ciliary rings. Scale bar=60 um.

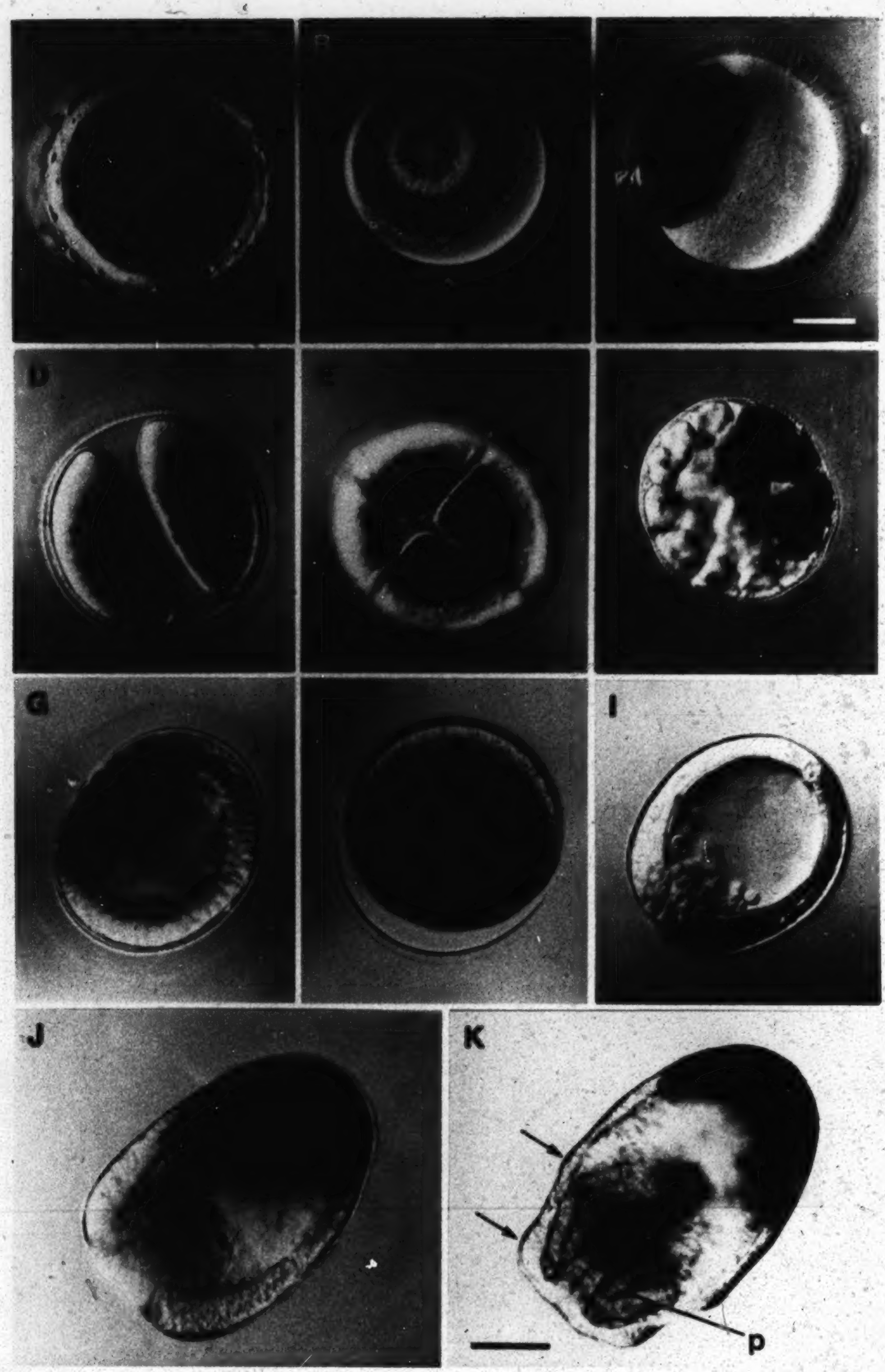
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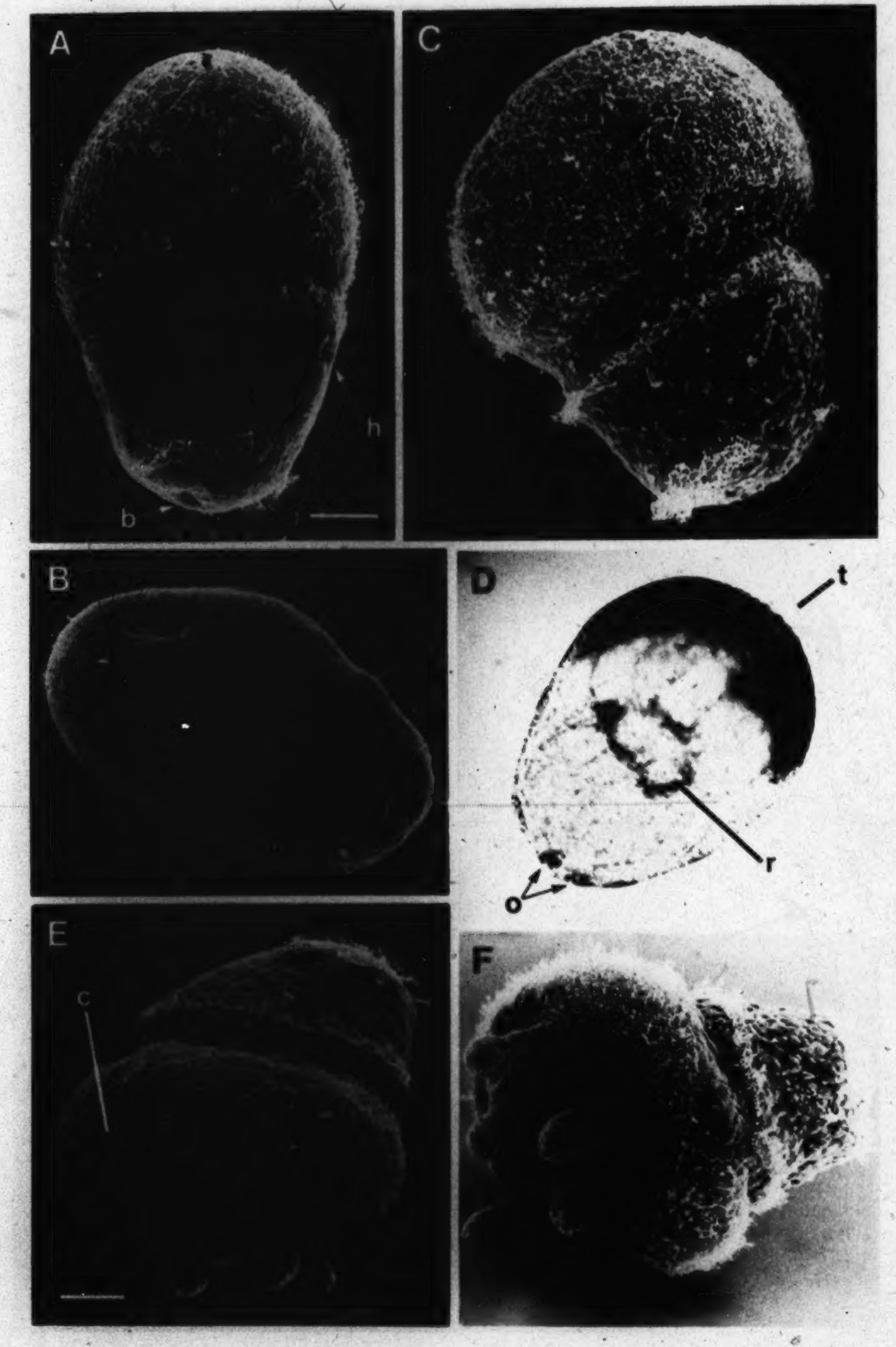
Table III-1. Chronology of development of Molpadia intermedia cultured at 11° ± 0.5°C at Friday Harbor Laboratories.

Developmental stage	time
fertilization	0 h
2-cell	2.25 h
4-cell	3.5 h
8-cell	4.75 h
16-cell	6 h
32-cell	7.25 h
64-cell	8.75 h
early blastula	13 h
spinning	23 h
hatching	24-25 h
gastrulation	32.25 h
late gastrula	46.25 h
early doliolaria	70 h
doliolaria	78 h
settlement	95-96 h
early pentactula	5.9 d

The coeloblastula (Fig. III-1G) is ciliated and begins to spin slowly approximately 0.5 - 1 hr before hatching; many blastulae displayed short, erratic, jerky movements just before hatching. As the blastula emerges from the broken membrane (Fig. III-1H), the mean spinning velocity increases to 10 revolutions/ 19.0 ± 1.5 sec (s. d., n=3), in a clockwise direction (viewed from the top or apical end). The process of hatching is probably accomplished through the use of a hatching enzyme, as is known for some echinoids, crinoids, and asteroids (reviewed by Davis 1968, 1981). The semi-transparency of the posterior two-thirds of the embryo permits observations of the development of internal structures in later embryonic stages. During this period, proliferation of mesenchyme cells is readily visible within the late coeloblastula from the vegetal plate, opposite the yolk cap (Figs. III-1I,J). Egression tracts (folds) are common in the blastular walls of Paracaudina (Caudina) chilensis embryos (Inaba 1930), but were not observed in blastulae of M. intermedia. Soon after hatching, the larvae become negatively geotactic and aggregate at the surface. Gastrulation is by invagination (Fig. III-1J). The archenteron begins to bend to one side and extends to two-thirds the length of the gastrula.

The embryo elongates and at 39 hr becomes bent to one side, tilting the cap of yolk. Further lengthening of the early larva straightens out the larval body and a small cleft in the posterior end is also evident at this time (52 hr). By 59 hr, 70-80% of the larvae have reversed their direction of swimming to counter clock-wise. Two posterior ridges created by the thickening of the epidermis distinguish the early (70 hr) doliolaria larva (Fig. III-1K); these epidermal ridges will later bear the ciliary rings. Two coelomic pouches are formed by outpocketings of the archenteron (Fig. III-1K) and were first noticed at 70 hr. The 77-hr larva measures 390 um in length and bears elongated cilia on the epidermal ridges (Fig. III-1K). A small ventral dimple slightly to the right of the anterior-posterior axis and positioned one-third of the body length from the apical end marks the site of the future vestibule. Loss of cilia on both sides of the epidermal ridges results in two rings of cilia, one encircling the still existing blastopore and the other situated almost midway on the larva (Fig. III-2A). The anterior third of the doliolaria is uniformly ciliated and contains the orangish-pink yolk. A faint sparkle from the first calcareous ossicle can be seen at 84 hr at the very tip of the posterior end; close to the yolk, a thin line of ossicles that will make up the calcareous ring becomes visible at 93 hr.

Fig. III-2. Molpadia intermedia. (A) Lateral view of 78-hr doliolaria showing two ciliary rings and ciliated preoral lobe, with hydropore (h) visible just posterior to the first ring of cilia; the blastopore (b) is still present. Scale bar=40 um. (B) Venter of doliolaria larva depicting vestibule (upper left) in transition from a ventral to an anterior terminal position. (C) Bulbous preoral lobe of the late doliolaria larva. (D) Light micrograph of late doliolaria o, posterior ossicles; r, calcareous ring; t, primary tentacle. (E) Protrusion of tentacles in early pentactula, with uniformly ciliated preoral lobe now forming a yolky collar (c). Scale bar=40 um. (F) Ventral view of pentactula just before loss of ciliation, showing further extension of the five primary tentacles.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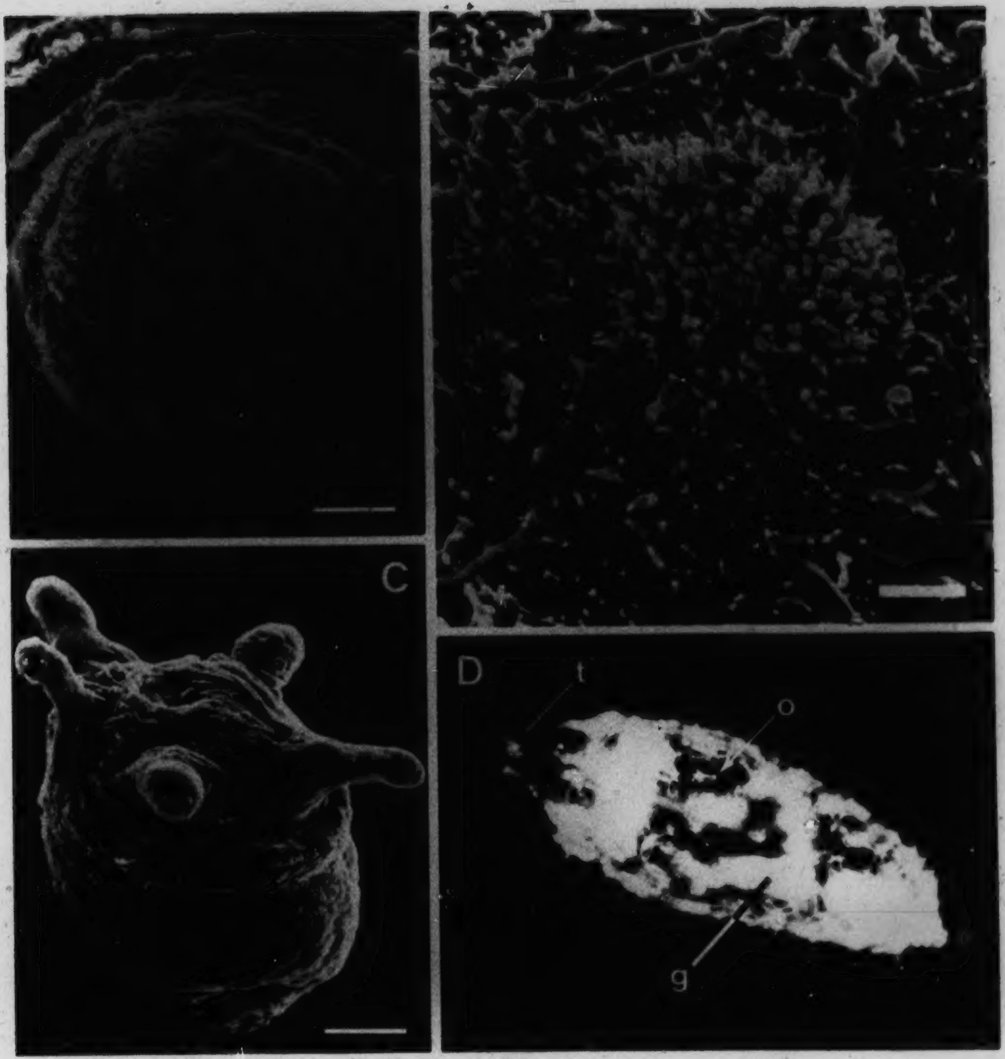
SETTLEMENT AND METAMORPHOSIS

The majority of doliolaria larvae, when 95 - 96 hr old, begin to settle on the bottom of the culture dishes where they swim around with apical end forward. The vestibule by this time has moved anteriorly into the yolky, ciliated lobe (Fig. III-2B). A reduction in the length of the larva begins at about four days, which rounds our the doliolaria to a length of 345 um and a width of 240 um.

Another reversal in swimming direction takes place a few hours into the sixth day of development, with the benthic larvae now swimming posterior end first. One prominent ossicle in the posterior end of the larva and the calcareous ring become very obvious at this time. In front of the thin calcareous ring, growth of the rudiments of the five primary tentacles enlarges the anterior half of the larva producing a bulbous appearance (Fig. III-2C). At 5 1/2 d, the primary tentacles are beginning to push out of the vestibule which is now almost at the very anterior end of the early pentactula (Figs. III-2D).

Larvae in other cultures kept in seawater tables were exposed to bright sunlight entering south-facing windows of the laboratory and displayed photonegative behavior, gathering on the dark side of the bottom of culture dishes. Many six-day old pentactulae rest on the bottom mud in a vertical posture (apical end up) with the fully extended tentacles being stuffed alternately into the center of the vestibule, spinning slowly to the right. Protrusion of the primary tentacles from the vestibule pushes back the mass of colored yolk that now takes the shape of a light pink collar underneath the tentacles, marked by the ciliated zone behind the tentacles in Figure III-2E. Pentactulae were observed at seven days congregated in the soft mud in small pockets of 50 - 75 individuals. By 8 1/2 days at a length of 290 um, 2 - 3 small ossicles can be seen in the region of the second ciliary ring. Covering the ends of the simple, digitate primary tentacles are areas where microvilli are concentrated into small tufts (Fig. III-3A). Figure III-3B shows the complexity of the microvilli occurring in and around the microvillar tufts. These tufts may function as sensory receptors for substratum selection by the settling larva. Cilia are lost from the seventh through the ninth day (Fig. III-3C), the pink yolk collar fades away, ossicles increase in number and size, and a very conspicuous pink gut appears.

Fig. III-3. Molpadia intermedia. (A) Tufts of microvilli on primary tentacle of pentactula. Scale bar=4 um. (B) Tuft of microvilli with associated network of long, elaborated microvilli on a primary tentacle. Scale bar=2 um. (C) Recently settled young that has resorbed the yolk collar and has lost external ciliation. Scale bar=50 um. (D) 70-d old juvenile. g, loop of sediment-filled gut; o, triradiate ossicles with spire; t, tentacle.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DELAY AND INDUCTION OF METAMORPHOSIS

To test for ability to delay metamorphosis, 100 - 150 pentactulae were reared in two bowls without mud. Five days after metamorphosis had taken place in control cultures, a small amount of mud from the adult habitat was added to both bowls. Within a period of 5 - 9 hr, larvae had begun congregating in small groups in the mud and by 10 1/2 hr had all settled. After 9 - 12 hr, the young had lost their external ciliation and pink yolk collar to become transparent, except for the small pink gut.

JUVENILE GROWTH

Continued growth of the five-tentacled juvenile in the laboratory involves further elaboration of the gut into a simple loop and proliferation of ossicles (Fig. III-3D). Small plates surround the anus in the 85-day-old juvenile; all other body wall ossicles anterior to these plates are larger and triradiate with a central spire, these spires give the young molpadiid a spiny appearance (Fig. III-3D). The completely transparent juveniles at this age are difficult to find in the mud; they lie on top of the mud or sit in the sediment with the oral end extended up to the surface, feeding on small particles which are also visible in the filled gut. The integument of 1 - 2 mm juveniles of Paracaudina (Caudina) chilensis is also translucent with ossicles in the anal region, but lacks ossicles elsewhere, except for the calcareous ring until it is 2 - 4 mm long (Kitao 1933). Tentacles of the three-month old M. intermedia are still five in number and terminate in a simple knob with no bifurcations. Kitao (1933) mentions that 10 tentacles are present by the time the juvenile of P. chilensis reaches a length of 2 mm, each tentacle being provided with two short terminal digits. In this species, tentacle bifurcation first occurs in the six-day old pentactula. No juveniles of M. intermedia were observed covered with adhering sand grains as is common with young of P. chilensis (Hozawa 1928; Inaba 1930; Kitao 1933).

D. DISCUSSION

As mentioned earlier, the only other study that has focused on molpadiid reproduction is that of Inaba (1930), who described the development of Paracaudina (Caudina) chilensis using histological methods. That species breeds in May and June in Japan and produces a brownish-red, 572 um diameter egg which floats. Tao (1930) found mature ova and spermatozoa present in April and May in the same species. Although Inaba (1930) characterizes the doliolaria larva of P. chilensis as bearing one ring of cilia, he mentions that another ciliary ring later encircles the anal field and he also depicts the two rings in his illustrations. Aside from the difference in size, the two-ringed doliolaria of Molpadia intermedia is essentially identical to this larva.

Observations of larval behavior under laboratory conditions are often difficult to interpret with respect to their ecological significance. The fact that cultured doliolaria larvae of M. intermedia began to settle at 80-85 hr and stayed on the surface of the mud for at least 3 1/2 - 4 d before metamorphosis might be explained as the time when molpadiid larvae in the plankton initiate their descent in the water column to adult habitats that may be as deep as 2,925 m (Clark 1907). Pentactulae of M. intermedia collected in plankton tows (M. Strathmann, personal communication) and this demonstration of delay of metamorphosis in the laboratory also suggest a period of several days or more when the larva is swimming downward to deep mud bottoms and searching for suitable habitat. The gregarious behavior of settling larvae in mud may explain the observed aggregations of adults in the field, although most adult echinoderm abundance distributions are thought to be determined primarily by post-settlement migration (Chia *et al*. 1984).

Apart from the work of Inaba (1930) and the present study, only fragmentary knowledge of reproduction exists for a few other molpadiid species. Gerould (1896), in probably the most thorough analysis of holothuroid morphology, examined the histology of the gonads and genital duct of Caudina arenata and described gametogenesis. Mature ova for this north Atlantic form measure 190 - 260 um and become ripe in early spring; mature spermatozoa with spherical heads were present from February through April. Spherical sperm heads are also found in Molpadia oolitica (Chia *et al*., 1975). "Very large ova" were seen in the ovaries of C. planapertura by Clark (1907) and eggs of 0.3 mm diameter were found in M. antarctica (Pawson 1969a). Hatanaka (1939) considered the breeding season of M. roretzii to occur in November or December in Japan, and Feral and Doumenc (1982) encountered Eumolpadia violacea (=Molpadia musculus) in the post-spawning state at the end of the austral summer (January-February) in the Kerguelen Islands. Eggs of Eupyrgus scaber ripen in August and are 250 um in diameter, this species has separate sexes (Heding 1935).

Of the four families in the order Molpadiida, the reproduction for one species in each of the two largest families has now been studied: Paracaudina (Caudina) chilensis in the family Caudinidae (Inaba 1930), has a circum-Pacific distribution and is probably abyssal (Pawson 1961); Molpadia intermedia in the family Molpadiidae (this study) also has a wide distribution, extending from Alaska to the west coast of South America, and a depth range that is mainly bathyal (7 - 2,925 m) (Clark 1907; Deichmann 1937; this study). These two molpadiid sea cucumbers are representative of the order with their widespread distributions in mud and sand habitats, and it is most likely that they are also typical of the entire order in their reproduction. In spite of the fact that the egg diameter of P. chilensis is more than twice that of M. intermedia, the reproduction and development of these two forms are very similar. In a taxon in which all members are dioecious (Clark 1907), and most show great similarity in body size and habits and are widely distributed over vast, uniform habitats, one would not expect to encounter varied modes of reproduction.

Clark (1907) states that adults of most molpadiids are longer than 50 mm, although the smallest (Eupyrgus pacificus) measures only 6 or 7 mm long. No brooding has yet been reported, but it is predicted that it may be found among the few exceptionally small-sized forms. Southern ocean molpadiids were characterized by Pawson (1977) as distinct from other shallow water holothuroids and echinoderms in lacking brood protection and conspicuous endemism. Molpadia antarctica is the only molpadiid recorded in the Antarctic fauna (Pawson 1969b), and from its egg size (0.3 mm, Pawson 1969a) we might infer the same pattern of reproduction met with in M. intermedia and P. chilensis.

The colored yolk was first noticed in September as a ring in the primary oocyte obtained from the ovary of M. intermedia, whereas eggs from a natural spawning in the laboratory in December lacked the ring and had, instead, a cap of colored yolk covering the animal pole. The yolk remains in this position throughout early embryonic development and is subsequently seen in the preoral lobe of larval stages. As part of metamorphosis, the pink yolk collar of the pentactula vanishes and is probably relocated to the gut region; the latter becomes conspicuous simultaneous with resorption of the collar and has the same pink color. The brownish-red tint of the larva of P. chilensis is also lost during metamorphosis and is retained only in the alimentary canal (Inaba 1930). This is similar to observations of other echinoderms in which cells of the preoral lobe in the larval stage are transferred into the juvenile digestive tract (Gemmill 1920; Chia 1968; Chia and Burke 1978).

The orangish-pink colored yolk in the animal half of the egg of M. intermedia is a unique character among holothuroids, with all other known holothuroid eggs being isolecithal. The pink cap of this telolecithal egg may serve as a distinguishing marker for studying the fate of the ooplasm as has been done in classical studies of experimental embryology using the pigmented egg of the sea urchin Paracentrotus lividus (Selenka 1883; Boveri 1901, both cited in Horstadius 1973).

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IV. DEVELOPMENT AND METAMORPHOSIS OF TWO PSOLID SEA CUCUMBERS, *PSOLUS CHITONOIDES* CLARK AND *PSOLIDIUM BULLATUM* OHSHIMA, WITH A REVIEW OF REPRODUCTIVE PATTERNS IN THE FAMILY PSOLIDAE[2]

A. INTRODUCTION

Psolid holothuroids comprise one of six families of sea cucumbers in the order Dendrochirotida (Pawson, 1982) and are some of the most unusual forms of echinoderms, being characterized by a flattened sole with a ring of well-developed ventrolateral tube feet that adhere to hard substrata and by an armored dorsum with large calcareous plates. Most psolids are morphologically adapted for living on hard substrata, mainly rock and shell, although algal fronds and holdfasts (Wootton, 1949; Deichmann, 1954; Pawson, 1964), coral (Deichmann, 1937, 1941), volcanic agglomerate (Pawson and Valentine, 1981), and shells of live cockles (Dakin, 1960) may also be employed as habitat substrata. These holothuroids are encountered primarily as subtidal epibenthos on the continental shelf, but some forms have extended their range into the low intertidal (Wootton, 1949) or to depths of 2800 m (Pawson, 1982).

Aside from taxonomic descriptions, published accounts on the biology of the Psolidae are few. Bullock and Dawson (1970) characterized the carotenoid pigments in *Psolus fabricii* and Fankboner (1978) described the functional morphology of psolid suspension feeding. As dendrochirotids, it is assumed that psolids are suspension feeders, although it is reported that part of the food of one species is acquired by deposit feeding (Miller and Pawson, 1984). More recently, Young and Chia (1982) studied the effects of larval settling and post-settling behavior on the spatial distribution of *P. chitonoides*. Brief descriptions of various brooding adaptations among the Psolidae are found in Thomson (1878a, b), Theel (1886), Ludwig (1897b, 1898), Clark (1901), Vaney (1906, 1907, 1914, 1925), Ekman (1925) and Miller (1983); partial descriptions of development are known for *P. phantapus* (Runnstrom and Runnstrom, 1918-1919; Thorson, 1946), *P. squamatus* (Agassiz, 1864; Agassiz and Agassiz, 1865) and for a

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Psolus sp. (Smith, 1977). A more complete but unpublished study on the early embryology of Lissothuria (Thyonepsolus) nutriens was carried out by Wootton (1949).

In the plankton-rich waters of the San Juan Archipelago, Washington, U. S. A., large beds of filter-feeding dendrochirotid holothuroids are frequently found on rock walls, boulder-cobble fields and mud bottoms; these dendrochirotids are usually dominant occupants of these habitats in terms of biomass and occupation of primary substratum. Psolus chitonoides and Psolidium bullatum, the subjects of this study, are the only abundant psolids encountered among the San Juan Islands. Psolus chitonoides, the larger of the two psolids, is usually reddish-orange with bright red tentacles, reaches a length of 7.5 cm, and may occasionally be seen intertidally at very low tides in crevices, under rocks or underneath overhanging ledges. In the San Juan Islands, this species is primarily subtidal in distribution and can occupy up to 100 % of the primary substratum on rock walls, large boulders and cobble; it is most abundant at 25 - 35 m depths in high current areas (Young and Chia, 1982; personal observation).

Psolidium bullatum is found in greatest densities (10 / 0.1 m^2) in San Juan Channel at depths of 13 - 35 m in areas of lower current speeds on the upper surfaces of large boulders covered with a thin (2 - 7 mm) layer of detritus and silt, leaving only the tentacles visible. This species is also common in some habitats alongside Psolus chitonoides at depths of 25 - 30 m on vertical rock walls and on top of, but never underneath, rocks and large boulders. Psolidium bullatum is only occasionally seen living at subtidal depths shallower than 11 m and has not been collected from the intertidal. Hadfield (1961) found this sea cucumber on mussel clumps (Modiolus modiolus), small rocks, and empty pecten shells dredged from 25 - 60 fathoms. This psolid is 2.6 cm in maximum body length, is colored pink to light lavender and is very low in profile, lying flat against the rock surface and preferring small depressions and corners. Both species are essentially sessile, but rare individuals of P. chitonoides have been seen crawling over rocks and hydroids.

Early embryos and larvae of Psolus chitonoides are one of the most obvious elements in the spring plankton of the San Juan Islands (Johnson, 1931; Johnson, 1932; Johnson and Johnson, 1950; personal observation). Fernald (1959) first mentioned the facility with which this species can be cultured in the laboratory and Jones (1960) studied the histologic details of some early developmental stages. Chia and Spaulding (1972) induced metamorphosis with tubes of Phyllochaetopterus sp. and Young and Chia (1982) presented a brief summary of development with their study of the behavior of the vitellaria and pentactula stages. Hadfield (1961) found ripe individuals of Psolidium bullatum in late spring months and mentioned "a period of little or no sexual activity" in the late fall and early winter. Aside from this, there is no information on the reproductive biology of Psolidium bullatum.

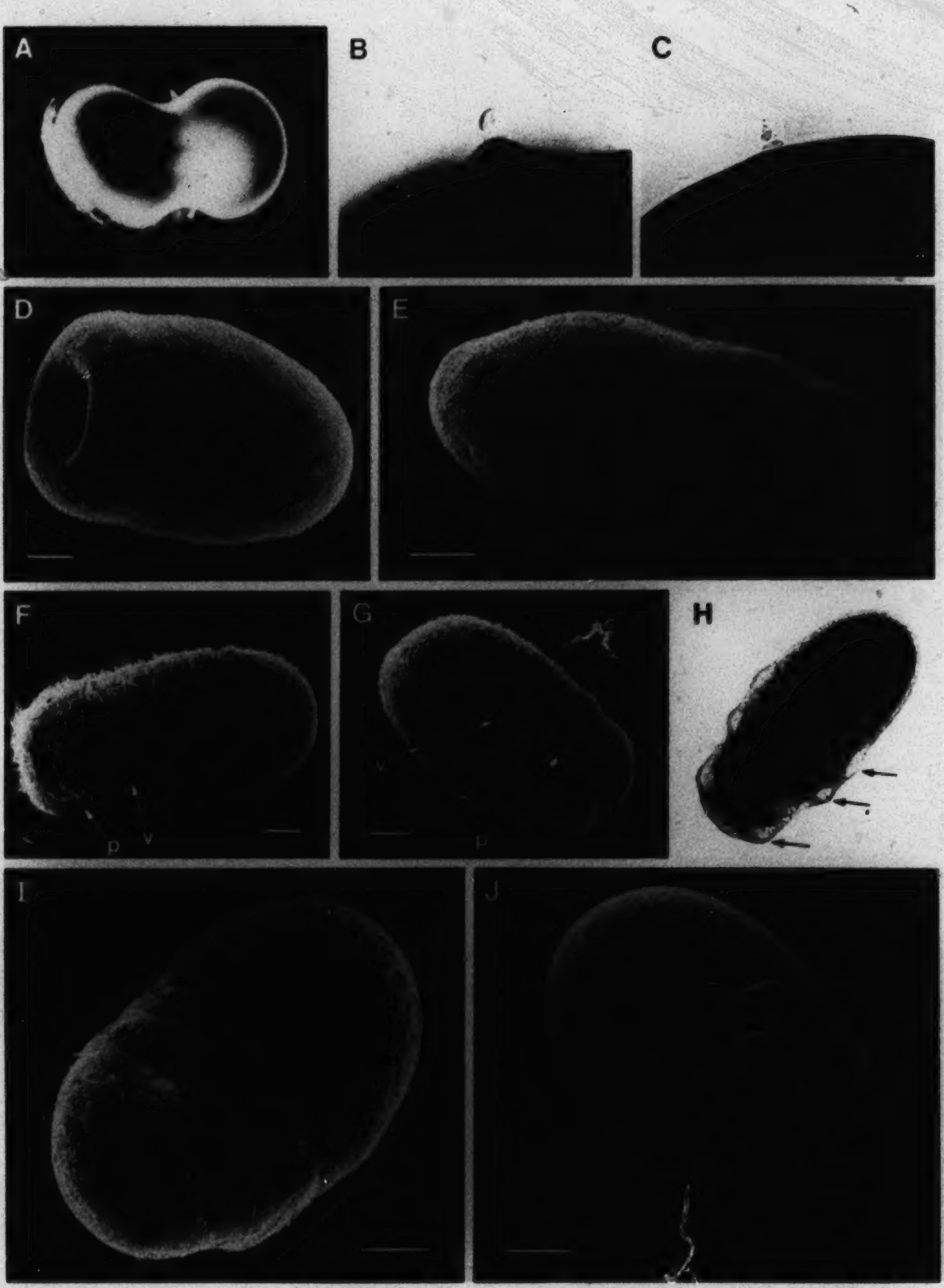
This paper presents the first information on the development of Psolidium bullatum. As well, a more complete picture of the development and metamorphosis of Psolus chitonoides is provided. Moreover, with so little known of psolid reproduction, a review of the literature on the reproduction of the entire family would seem important to help clarify reproductive trends and adaptations in a taxon that is so highly specialized in its body form (Pawson, 1966).

B. MATERIALS AND METHODS

Individuals of Psolidium bullatum were initially dredged at Iceberg Point, Lopez Island, Washington (48° 25' N. and 122° 54' W.) in April 1981. Collections and observations of both species using SCUBA were made from August 1981 through April 1984 in San Juan Channel (48° 33' N. and 122° 59' W.) at a depth of 25 - 30 m; specimens were kept in running seawater tables at the University of Washington Friday Harbor Laboratories. Observations of sea cucumber egg abundance in the surface plankton were made from the laboratory dock and during SCUBA dives.

Individuals were placed in aquaria and in separate culture bowls in seawater tables to examine spawning behavior and to collect gametes. The only instances of evisceration were with Psolus chitonoides when extremely stagnant conditions prevailed, resulting in the digestive tract being thrown out the oral end (sometimes anally) and gonads pushed out through the anus. Eggs stripped from ripe ovaries of P. chitonoides began to escape from the follicular layer 30 - 60 min after removal from the ovaries (Fig. IV-1A). Attempts to fertilize these stripped eggs were unsuccessful. Spawning in both species occurred frequently in the water tables and could occasionally be induced by either turning off water circulation in the seawater table or by removing bowls containing sea cucumbers from the seawater table and allowing them to warm from water temperatures of 9 - 11°C

Fig. IV-1. Psolus chitonoides. (A) Ovulation of oocyte removed from ripe ovary, showing oocyte escaping from layer of follicle cells; (B) first polar body and protuberance on oocyte surface; (C) appearance of second polar body at 10 - 50 min after fertilization. (D) 48-hr gastrula. Scale = 50 um. (E) Elongate, uniformly ciliated larva (65 hr). Scale = 100 um. (F) Early ciliated larva showing the first signs of vestibule (v) and podial pit (p) formation. Scale = 100 um. (G) Early doliolaria larva with the enlarging vestibule (v) and a podial pit (p) just posterior to the vestibule. Scale = 100 um. (H) Light micrograph of early doliolaria; arrows indicate the three epidermal ridges where the three ciliary rings will later form; the darkest opaque region is the yolky preoral lobe. (I) Dorsal view of 7-d doliolaria with three distinct ciliary rings and an evenly ciliated preoral lobe; the hydropore is also visible between the second and third rings. Scale = 100 um. (J) Emergence of the five tentacles from the ventral vestibule in the 7-d doliolaria; between the second and third ciliary rings are the two podial pits of the primary podia. Scale = 100 um.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to 15 - 17° C for 1 - 2 hr. Fertilized eggs were obtained from natural spawnings in seawater tables and from artificial fertilizations. For artificial fertilization, 0.5 ml of dilute sperm (one drop "dry" sperm from spawning males, or from ripe testes, diluted with 50 ml of 0.45 um mesh size Millipore-filtered seawater) was added to every 50 ml of seawater with 150 - 200 spawned eggs; after 10 min, eggs were rinsed several times with filtered seawater. Fertilized eggs (100 - 200) were cultured in glass bowls with 300 ml of filtered seawater and maintained in an incubator at 11° ± 0.5° C, with a 12-hr photoperiod using flourescent light for uniform temperature and light conditions. Additional cultures were also kept in seawater tables. Incubated embryos from at least two culture bowls from each of 3 - 4 females of each species were monitored for development schedules. Timing of developmental stages was determined when approximately 50% of the incubated embryos or larvae had reached a particular stage.

The materials and procedures used to prepare embryos and larvae for scanning electron microscope study were the same as outlined in chapter three.

C. RESULTS

GONADS, GAMETES AND SPAWNING

Gonads of Psolus chitonoides and Psolidium bullatum are morphologically similar in both, tufts of tubules lie slightly left of the anterior-posterior axis and occupy a substantial portion of the coelomic cavity when ripe. Ripe ovaries of P. chitonoides are dark red, sometimes a reddish brown, and the ripe testes light pink or white; the coloration of mature ovaries and testes of P. bullatum are tan and white, respectively. Sexes can often be distinguished in ripe adults by the color of the gonad that shows through the sole, particularly the thin, semi-transparent sole of P. bullatum.

The spawning period for Psolus chitonoides normally begins during the first and second weeks of March in the field and continues through the second and third weeks of May, with occasional light spawning preceding and following this main period. Heaviest spawning takes place on sunny days (chapter two) for P. chitonoides. No sightings of spawning in the field for Psolidium bullatum have been made by us or, to my knowledge, recorded by other divers. If the timing and frequency of laboratory reproductive behavior is a reliable indicator of reproductive activity in the field, as demonstrated by P. chitonoides, I would predict the most intense interval of spawning for P. bullatum to last from the fourth week of March through the first week of May, based upon the recorded spawnings of 106 adults of this species in the laboratory.

Spawning could be induced in both sexes of Psolus chitonoides sometimes one month before the normal spawning season. Male and female individuals of P. chitonoides isolated in separate bowls, both spawned without the influence of "egg water" or sperm. Psolidium bullatum did not respond as readily to artificial methods.

Males of both species usually spawn first. It is common for most males in the laboratory to release sperm on a number of different days and in some cases (for Psolus chitonoides) to emit sperm on 2 - 3 different occasions during a single day. Sperm is of the primitive type for both P. chitonoides (Chia et al., 1975) and Psolidium bullatum. Both sexes of P. chitonoides, if disturbed during spawning, resume their release of gametes within 2 - 15 min; P. bullatum is not as quick to recover when disturbed and will frequently remain with tentacles withdrawn and will not recommence spawning for 30 - 60 min.

A long rope of eggs is produced by female Psolus chitonoides, held together by a transparent mucoid material, that floats to the surface where it breaks apart. The eggs are bright red and positively buoyant. The egg measures 627 ± 5 um (s.e.), in length (n=50, 10 eggs measured from each of five females) and is surrounded by a jelly coat 60 um thick; maximum measured fecundity was 34,700 (of 19 fecundity estimates from natural spawnings of females in the laboratory). Eggs of P. chitonoides are present in greatest numbers in the surface waters for a duration of 1 - 3 d following intervals of clear, sunny weather; these brightly colored, floating eggs are one of the most conspicuous members of the spring plankton. Infrequently, a female would also release globular material with the eggs in the rope, of the same color as the eggs. In dissected individuals, the normally cylindrical-shaped ripe ovaries were on occasion seen abnormally distended with this globular material. Gerould (1896), Theel (1901) and Ohshima (1925) also encountered irregular, rounded masses of yolk in the ovaries of Caudina arenata, Mesothuria intestinalis and Sclerodactyla (Thyone) briareus, respectively, that were considered to be degenerating oocytes.

Females of Psolidium bullatum discharge up to 3,100 golden yellow to light brownish orange eggs of 330 ± 4 um (s.e.) length (n=20, 10 eggs measured from each of two females) that are slightly negatively buoyant. A loose mucus is normally spawned with the eggs.

DEVELOPMENT OF PSOLUS CHITONOIDES

Cleavage type and pattern for the large opaque eggs of Psolus chitonoides are equal, holoblastic and radial with the first two divisions along the animal-vegetal axis and the third cleavage horizontal with subsequent cleavages alternating between the two cleavage planes. Many cultures had approximately 40 - 80 % of the embryos with slightly subequal cleavage (also noted by Jones, 1960). Time of extrusion of the second polar body occurred anywhere from 10 - 50 min after fertilization (Fig. IV-1C). Wrinkled blastulae appear at 18 hr (Table IV-1) and begin smoothing out by 24 hr, hatching takes place, releasing a ciliated blastula that rotates in a counter clockwise direction. The first external signs of vegetal plate formation become apparent with a lightening in color of the periphery of the vegetal half, reaching a light yellow as the vegetal half flattens, the blastopore forms by invagination at the center of the vegetal plate (Fig. IV-1D) as early as 44 hr in some embryos. Subsequent to vegetal plate formation, mesenchyme cells are released into the blastocoel from the vegetal plate as well as from the inner surface of the ectodermal wall (Jones, 1960).

Gastrulae begin to elongate at 60 hr, sometimes to a maximum length of 1125 um, into a uniformly ciliated larva (Fig. IV-1E). The blastopore commences to close over (Fig. IV-1E) and a small, ventral, crescent-shaped, ectodermal invagination, slightly to the right of the central axis, marks the beginning of the vestibule (Fig. IV-1F). The region of the larva anterior to the vestibule is the preoral lobe. Also accompanying the appearance of the vestibule is a small posterior indentation, the podial pit of the future left primary podium (Figs. IV-1F, G).

Reduction in length (to 925 um) along with an increase in width begin the transformation of the uniformly ciliated larva into a doliolaria. Three posterior ridges formed on the early doliolaria at 80 - 90 d giving the appearance of three ciliary rings, but ciliation is still uniform over the entire larval surface (Fig. IV-1H). The vestibular pit, now

Table IV-1. Development chronology of Psolus chitonoides and Psolidium bullatum reared at 11° ± 0.5°C (after fertilization) at Friday Harbor Laboratories.

stage	Psolus chitonoides	Psolidium bullatum
first cleavage	2h	3 1/2h
second cleavage	3 3/4h	5h
third cleavage	5h	6 1/2h
fourth cleavage	6 1/4h	8h
fifth cleavage	8h	9h
sixth cleavage	10 1/4h	10 1/4h
blastula	18h	19 3/4h
hatching	--	28 1/2h
gastrula	40h	40h
early larva (uniformly ciliated)	60-70h	55h
early doliolaria	80-90h	70-75h
late doliolaria	6 3/4-7 1/4d	4 1/2-4 3/4d
early pentactula	8 1/2-9d	7-7 1/4d
settlement	11-12d	7 3/4-8 1/2d
sole formation (completion of metamorphosis)	26-32d	?

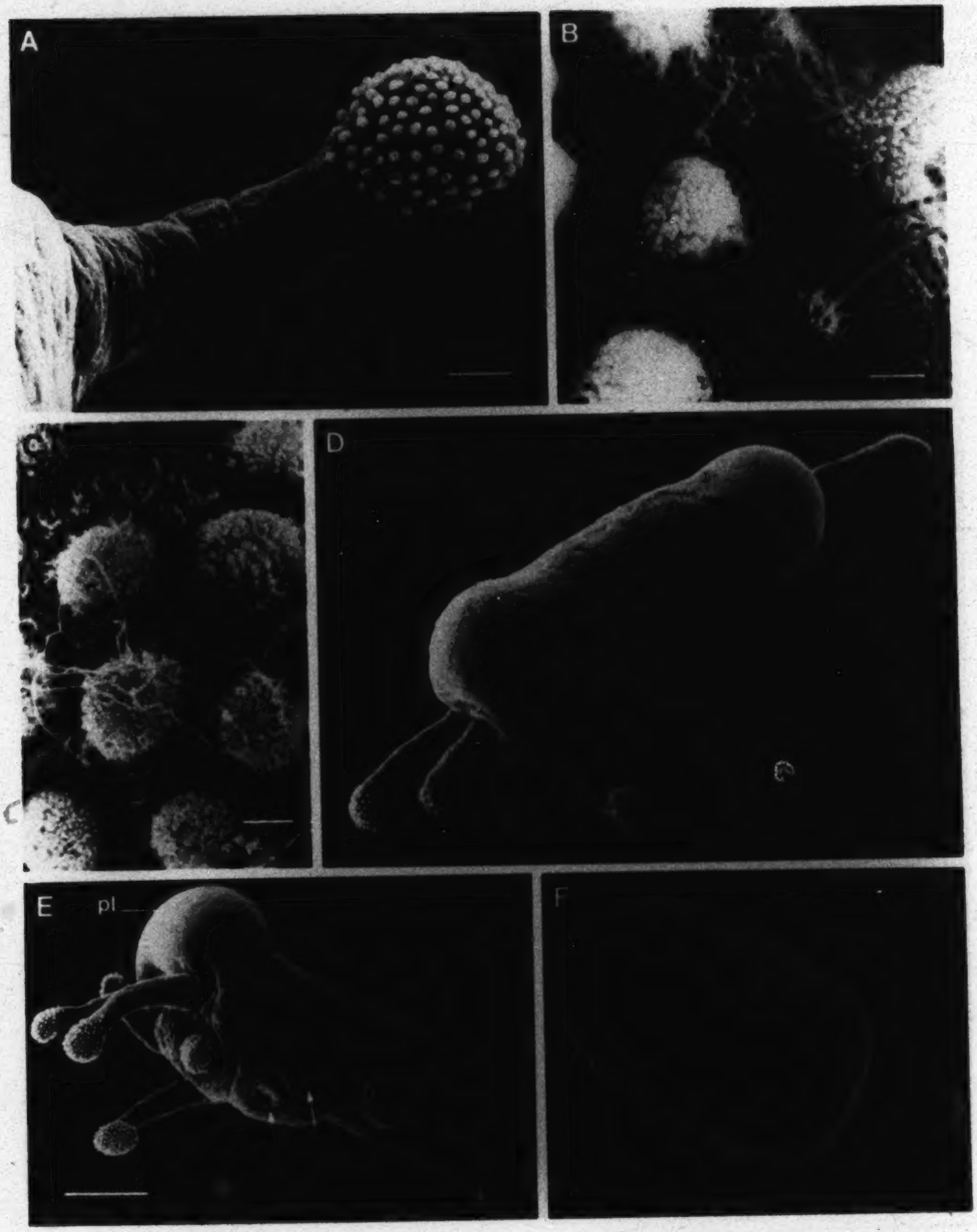
positioned just anterior to the first ciliary ridge, continues to enlarge.

Shortly thereafter, another podial pit becomes visible in the posterior third of the larva; it is also positioned between the second and third ciliary rings, but laterally and 90 degrees to the right of the podial pit that is directly posterior to the vestibule. Cilia between the three ridges and on the posterior end of the larva are then lost, leaving three distinct rings of cilia and a completely ciliated preoral lobe (Fig. IV-1I, J). These distinct rings characterize the typical dendrochirotid larval form, the doliolaria. During the early and late doliolaria stages, it is difficult to determine with the light microscope whether the larvae still retain or have lost the cilia in between the rings. The vestibule continues to enlarge and the five primary tentacles become visible and begin to protrude from the vestibule (Fig. IV-1J). The mouth, however, has not yet formed by this time. In the doliolaria, the podial pit on the right side gradually assumes a ventral position alongside the other pit (Fig. IV-1J). With formation of the three ciliary rings, the doliolaria begins vertical swimming to the bottom of the culture bowl and back to the surface. In this phase of vertical swimming,the preoral lobe is reddish orange and the region posterior to the vestibule is yellow. The preoral lobe is always maintained upward (probably due to a large store of lipid) while swimming and the spinning direction of almost all larvae remains clockwise (when viewed from the apical end). Faint glints of the first ossicles appear in the late doliolaria towards the end of the vertical swimming stage.

During the vertical swimming phase, the five primary tentacles begin to elongate and readily adhere to surfaces with which they come into contact; the larva at this time frequently attaches with the tentacles to the bottom of the dish for varying lengths of time. The dorsal hydropore has made its appearance by this time. The mean swimming rate for advanced doliolaria larvae swimming downward was 0.067 ± 0.016 cm/sec (s.d., _n_=10); upward swimming (or rising) rate was more than twice as fast at 0.142 ± 0.027 cm/sec, (s.d., _n_=10). After the larva settles, the cilia disappear and the two primary podia emerge from the podial pits and elongate.

The individual with five primary tentacles and two primary podia becomes permanently benthic after loss of the cilia; occasional larvae were noted to protrude the primary podia before settlement. Hyaline papillae and short cilia (Figs. IV-2A, B) cover the knobbed ends of the primary tentacles and are present from the time the tentacles come

Fig. IV-2. Psolus chitonoides. (A) Primary tentacle of pentactula with knobbed end covered with papillae. Scale = 25 um. (B) The surface of papillae on primary tentacles is composed of numerous micropapillae; short cilia are also present in between papillae and usually occur in pairs. Scale = 2 um. (C) Surface of primary tentacle with microvillar strands and globules strung out over and among the papillae. Scale = 2.5 um. (D) Settled pentactula after loss of cilia. (E) Receding preoral lobe (pl) and newly forming secondary podia (arrows) on the pentactula. Scale = 225 um. (F) Dorsal view of pentactula with diminishing hydropore (arrow).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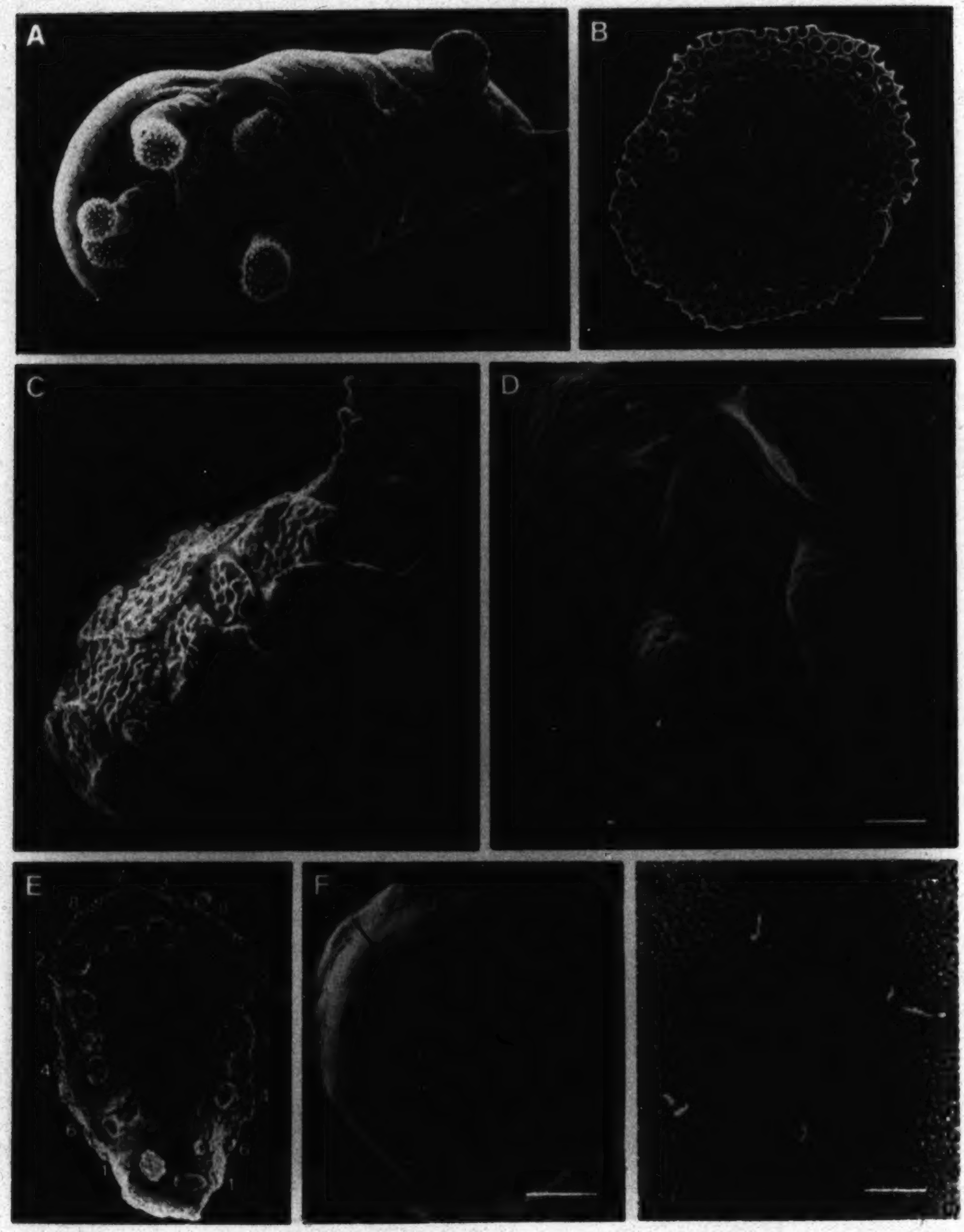
to view within the vestibule. These papillae were also noted by Johnson (1931). Higher magnification with SEM reveals numerous micropapillae covering each papilla (Figs. IV-2B, C); a similar arrangement of papillae and cilia is known for the adult tentacle (Fankboner, 1978). Also on the tentacle surface among the papillae are numerous elaborate microvilli that appear as strands and small globules (Figs. IV-2B, C). Short cilia are also found on the flat bottom surface of the primary podia. The negatively phototactic pentactula walks over the hard substratum, using the sticky tentacles and podia to attach to surfaces (Fig. IV-2D). The first pair of secondary podia emerge in the late pentactula stage just prior to formation of the sole (Fig. IV-2E) and the dorsal hydropore reduces in size (Fig. IV-2F). From the time of emergence just before settlement, the tentacles display the normal dendrochirotid behavioral rhythm of randomly stuffing the tentacles into the region of the future mouth even one at a time even though the mouth has not yet formed (Fig. IV-3A).

Recently settled larvae were examined to determine whether they were positively or negatively buoyant. If the larvae displayed no swimming behavior when detached from the bottom of the bowl and then pipetted into the water column, they were classed as permanently settled. Twenty six permanently settled larvae in a bowl were then warmed over a hot plate for 1 min and then the adhesive tentacles and podia of the dead larvae were loosened from their attachment to the dish bottom. All but three individuals floated to the surface, apical end (reddish orange in color) up; the larvae were examined and those not floating were discovered to have large ossicles.

Soon after settlement, there is rapid formation of ossicles simultaneous with the resorption of the preoral lobe. No ossicles form within the preoral lobe. As the preoral lobe recedes, the primary tentacles move from their ventral position to the anterior position seen in adults. The most obvious events during this final phase of psolid metamorphosis are the formation of large calcareous plates (Fig. IV-3B) that cover the dorsal bivium (ambulacral radii C and D) (Fig. IV-3C) and the flattening of the ventral trivium (ambulacral radii A, B and E) to form the sole. The settled pentactula adheres initially to the substratum with the five primary tentacles and two primary podia. At 29 d, there is still no branching of the tentacles nor growth of new or secondary tentacles. The first bifurcations of the tips of the three dorsal tentacles occur at 30 - 33 d, while the two ventral tentacles retain their pad-like appearance (Fig. IV-3D). By 20 d, a few pentactulae

Fig. IV-3. Psolus chitonoides. (A) Antero-ventral view of pentactula; no mouth has formed yet. (B) One of the large ossicle plates that cover the newly metamorphosed juvenile. Scale = 50 um. (C) Young juvenile (1.2 mm long) with dorsal plates; three plates surround the anus. (D) Early branching of the three dorsal tentacles in the early juvenile; arrows point to the two ventral tentacles that remain pad-like in shape long after the other three primary tentacles have undergone extensive growth and branching. Scale = 50 um. (E) Sole of young juvenile with 14 podia on ambulacral radii B and E. Numbers represent order of appearance of the podia; podia with the same number emerge simultaneously. The first two primary podia denote the posterior end. (F) View of the bottom surface of a tube foot of a juvenile. Edge of zone of differentiated epidermis indicated by arrow. Scale = 20 um. (G) Magnification of bottom of tube foot of juvenile, showing presence of short cilia. Scale = 5 um.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possess large ossicles and are just beginning formation of the sole. With deposition of the dorsal calcareous plates and the dorso-ventral flattening, the first pair of secondary podia form by 29 d anterior to the two primary podia and slowly move outwards, remaining on the edge of the expanding sole. A fifth podium becomes visible at 31 - 32 d on the anterior end of the sole, slightly to the left of the midline (ambulacral radius A). The usual appearance of podia, thereafter, resumes in pairs with one podium on either side of the midline opposite to the other on ambulacral radii B and E. Figure IV-3E depicts the position and order of appearance of the 14 podia present at 54 d. A circular zone of differentiated epidermis and scattered short cilia are visible on the bottom surface of tube feet of pentactulae and early juveniles (Figs. IV-3F,G). Viewed ventrally, new tube feet can be seen forming internally (before they emerge) as clear areas in the orange yolk ringing the interior edge of the sole. If these juveniles are dislodged and turned over, they immediately attach the tentacles to the substratum and flip themselves over, regaining their original stance. Mean length of the 54-d juvenile is 1259 ± 32 um (s.d., n=5), measured from the posterior end of the sole to the anteriormost oral plate.

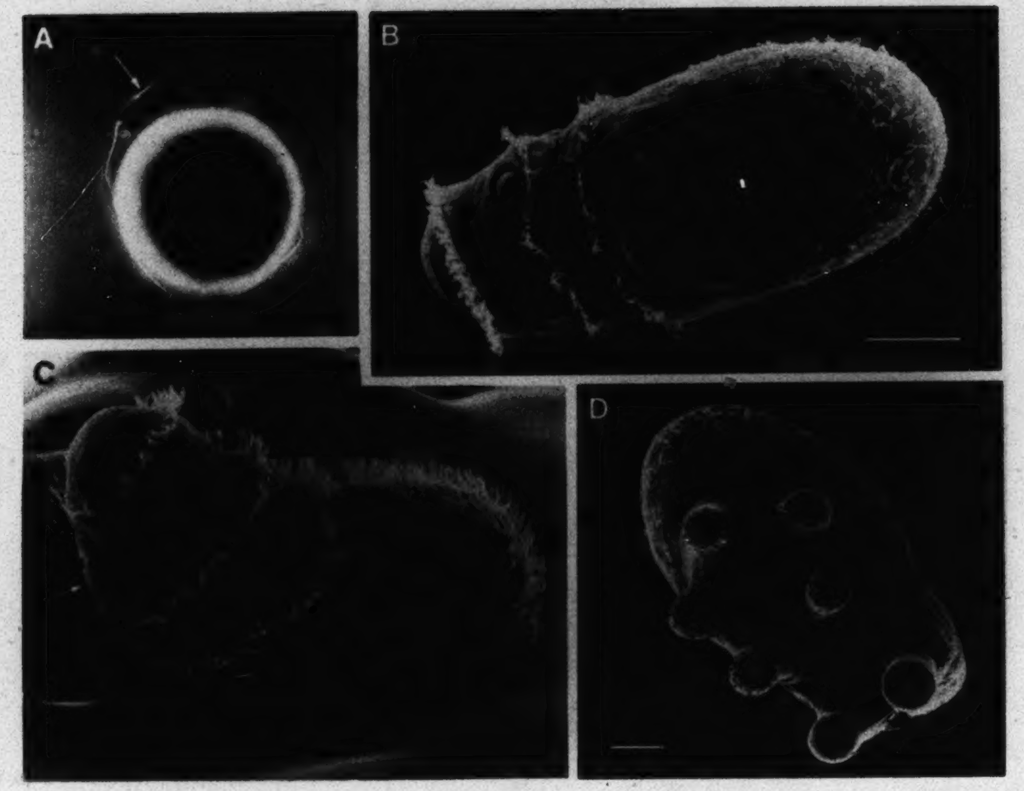
Larvae settled readily on nearly all hard surfaces; some attached their primary tentacles to the culture water surface tension and completed a normal metamorphosis upside down. Young and Chia (1982) demonstrated that the pentactula settles gregariously on adults and that late larvae and early juveniles are strongly photonegative. In additional bowls under very crowded conditions (300 - 400 individuals), instances of larvae coalescing were noted, but most went through an extended metamorphosis that, in some cases, lasted up to eight months before the dorsal armor and sole were complete. In 10 - 12 individuals, ossicles grew to a large size, but after a period of 1 yr, no flat sole had yet been established. In cultures supplied with algae and fine detritus, juveniles obtained their food by deposit feeding on the bottom of the dish. Fecal boluses were first noticed at 50 - 52 d. Juveniles kept in unfed cultures were still alive at the end of one year, were no longer than 1.2 mm and had not added new tentacles to the original five primary ones. Ossicles were larger and more numerous and the looped gut was visible through the light orange, semi-transparent body wall.

DEVELOPMENT OF PSOLIDIUM BULLATUM

Development in this species is very similar to that of Psolus chitonoides, with some differences in morphology, behavior and timing of developmental stages (Table IV-1). The embryo of Psolidium bullatum is smaller and less opaque than the embryo of Psolus chitonoides, facilitating observations of early developmental events. The schedule of early cleavages is close to that of P. chitonoides. In the late coeloblastula (25 hr), there is a thickening of the cells at the vegetal pole; these cells are easily distinguished by their light yellow color in contrast to the more opaque light tan-orange color of the rest of the embryo. Once the egg membrane is broken, the spinning blastula takes about 1 1/2 min to leave the surrounding membrane and jelly layer (Fig. IV-4A). By 35 hr, the embryo elongates to 400 um and is egg-shaped; the early gastrula forms 5 hr later with continued thickening and flattening of the vegetal plate, giving the embryo a rounded cone shape. The posterior of the gastrula becomes less opaque and whitish in color by 51 hr at a length of 410 um. Larvae congregate side by side in groups of 3 - 50 individuals, in a vertical position with the orange pigmented (apical) end up; some swim along the dish bottom in a horizontal position, spinning as they go. Of five larvae, the fastest spinning rate measured was 1.03 rev/sec (revolutions per second; median=1.12 rev/sec, range=1.03 - 1.26 rev/sec). Further lengthening to 430 um changes the larva into a long cylindrical shape that has a slight flattening of the venter; the first sign of the developing vestibule is barely visible as a slightly differentiated circle bordering the lower ventral edge of the pigmented region. The spinning increases in speed to one revolution per 0.82 sec (median=0.91 rev/sec; range=0.82 - 1.11 rev/sec) when the larva reaches a length of 485 um; the larva reverses its direction of spinning (to clockwise) at 65 hr and swims at the surface.

The early doliolaria is characterized by three ciliary ridges that encircle the posterior half of the larva at 70 hr. The vestibule opens by 89 hr and the early doliolaria measures 600 um long. A circular depression for a primary podium is evident at 4 d, directly posterior to the vestibule and between the second and third ciliary rings. Small brown splotches and streaks of various shapes can be seen in the posterior third of the body. The primary podia protrude from the vestibule (Fig. IV-4B) and swimming behavior is up and down in a vertical position similar to Psolus chitonoides, with some larvae swimming horizontal to the bottom, apical end forward. The older doliolaria decreases in

Fig. IV-4. Psolidium bullatum. (A) Hatching blastula at 28.5 hr; arrow points to detached membrane. (B) Three-ringed doliolaria with emerging five primary podia and two posterior podial pits. Scale = 75 um. (C) Normal position of larva at settlement. Arrow indicates a primary podium beginning to push out; opposite the ventral podium and just posterior to the second ring of cilia is the small dorsal hydropore. (D) Ventral view of recently settled pentactula with some cilia still remaining on the preoral lobe. Scale = 60 um.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length to 550 - 560 um, with some doliolaria larvae as small as 475 um. This shortening also results in a wider larval body.

Details of settlement are like those for Psolus chitonoides, with larvae occasionally attaching to the bottom in the typical posture shown in Figure IV-4C. The primary tentacles of Psolidium bullatum, although proportionately much shorter than tentacles of pentactulae of P.chitonoides, are very prominent at settlement and occupy a substantial area of the ventral surface of the 7-d old, 445 um-long larva (Fig. IV-4D). Settling larvae and settled pentactulae of both psolids maintained in cultures in seawater tables were subject to bright sunlight entering the windows on the south side of the laboratory and displayed photonegative behavior, aggregating in the bottom of the bowls opposite these windows. Incubated cultures showed no such behavior, owing to the even illumination of the incubator. Larval swimming behavior appeared more consistent for incubated larvae than for larvae kept in seawater tables; culture bowls of the latter were only partially submerged and subject to temperature gradients and fluctuations, particularly during warm days.

Shortly after settlement, the two primary podia increase in length and attach to the substratum. Cilia are first lost from the ciliary rings and then from the preoral lobe. Ossicles become visible posterior to the preoral lobe at 8 1/2 d; the posterior two thirds of the body later becomes more transparent and the preoral lobe a light yellowish orange, with individuals possessing many large ossicles posteriorly at 14 1/2 d. By this time, numerous ossicles have formed in the primary podia, but only a few in the tentacles. Resorption of the preoral lobe is a gradual process that continues until approximately 22 d, when the individuals (size=670 um) are well armored with a coat of ossicles and have very little preoral lobe remaining.

No secondary podia, additional tentacles, nor branching of the primary tentacles had occurred at the end of 36 d. Branching of the three dorsal primary tentacles begins at 5 1/2 months.

Pentactulae were provided rock, conspecific adults, adult Psolus chitonoides, and tubes of spionids and Phyllochaetopterus sp., but there was no complete metamorphosis to the flattened juvenile form as in P. chitonoides, even in cultures maintained for 1 yr. Although embryo cultures from 3 - 5 females of Psolidium bullatum were obtained each breeding season for four consecutive years (1981-1984) and the larvae reared through settlement, no success was achieved with attempts to induce metamorphosis. Individuals kept for 1 yr in bowls with no food were still alive and had the tentacles, podia and body completely covered with ossicles. In addition to the original two primary podia, six individuals had grown 3 - 4 dorsal podia; one individual has eight tentacles with only simple bifurcations. Those provided polychaete tubes fed on the detritus and microflora on the tubes and defecated boluses.

D. DISCUSSION

Brooding for five species of psolids was discussed by Ludwig (1904) and Vaney (1925) in their early reviews of holothuroid broodcare. More recently, Hyman (1955) and Boolootian (1966) have briefly summarized breeding seasons and brooding for 9 - 10 psolid species. Table IV-2 summarizes the available information from published literature on sexual reproduction in the family Psolidae. Not included in the table are egg sizes of 200 um for Psolus neozelanicus (Mortensen, 1925), "less than 500 um" for Lissothuria mortenseni (Pawson, 1967), and 0.2 mm for Ekkentropelma brychia (Pawson, 1971), since it was not known whether or not the oocytes examined were mature. The only report of any type of asexual reproduction in this family is transverse fission in P. valvatus, where each half of the split sea cucumber regenerates the lacking parts (Mortensen, 1927).

The most obvious characteristic shown in Table IV-2 is the prevalence of brooding, with 9 of the 13 species exhibiting broodcare. Four different modes of brooding are found: 1) on the dorsum, 2) on the ventral sole, 3) around the crown of tentacles, and 4) internally. Dorsal tube feet of Lissothuria (Thyonepsolus) nutriens retain developing embryos in external dorsal depressions (Wootton, 1949) and Psolus ephippifer possesses brood chambers beneath the dorsal calcareous plates (Thomson, 1878a, b; Theel, 1886). Large interradial pouches surrounding the tentacular crown house the broods of P. koehleri (Vaney, 1914). Young can be found attached to the ventral sole, as in P. antarcticus (Ludwig, 1897b, 1898), or with the embryos in pockets, folds, or swellings of the sole as is known for Psolidium incubans (Ekman, 1925), Psolus figulus (Ekman, 1925), P. granulosus (Vaney, 1907) and P. punctatus (Ekman, 1925). Miller (1983) recently

Table IV-2. Summary of information on reproduction in the holothuroid family Psolidae. Dashes signifies no data available.

Species	Max. Adult Size(mm)	Breeding Season	Egg Size (um)	Reproductive Pattern	Geographic Distribution and Habitat	References
Lissothuria antillensis	15	continuous	400	brooder (coelomic)	Bermuda, Caribbean; under rocks	Miller, 1983, 1985
L. (Thyonepsolus) nutriens	26	Feb-Dec (continuous)	560-600	brooder (dorsal depressions)	California; kelp holdfasts and kelp bed, rock	Clark, 1901, 1902; Deichmann, 1941; Wootton, 1949; MacGinitie&MacGinitie, 1949
Psolidium bullatum	26	Mar-May	330	pelagic larva (doliolaria)	No. Pacific; rock walls, shells, mussels, cobble fields, boulders	Hadfield, 1961; this study
P. incubans	12	---	640 (young?)	brooder (depressions in sole)	Antarctic; littoral	Ekman, 1925
Psolus antarcticus	55	---	600-700	brooder (under sole)	So. Atlantic, circumpolar-Antarctic; rock	Theel, 1886; Ludwig, 1897b, 1898; Pawson, 1969a, b
P. chitonoides	75	Mar-June	600-627	pelagic larva (doliolaria)	No. Pacific; rock walls, cobble fields, boulders	Johnson, 1931; Johnson&Johnson, 1950; Fernald, 1959; Jones, 1960; Young& Chia, 1982; this study
P. ephippifer	40	Jan, Feb	---	brooder (chambers	Antarctic: coarse gravel, sand, mud	Thomson, 1878a, b; Theel, 1886; Ludwig,

				underneath, dorsal plates)		1897a, 1898; Pawson, 1969b
P. (*Cuvieria*) *fabricii*	100	---	---	pelagic larva	No. Atlantic; under stones	Agassiz, 1864; Agassiz&Agassiz, 1865; Deichmann, 1930
P. *figulus*	9	---	640	brooder (depressions in sole)	Antarctic; sand	Ekman, 1925
P. *granulosus*	22	---	500	brooder (folds of sole)	Antarctic; shore	Vaney, 1906, 1907
P. *koehleri*	65	---	---	brooder (interradial pouches surrounding tentacular crown	Antarctic; rock, gravel, mud	Vaney, 1914, 1925; Pawson, 1969b
P. *phantapus*	150	Feb to midsummer	500-600	pelagic larva	No. Atlantic; stones, shells	Mortensen, 1901, 1927; Mortensen& Lieberkind, 1928; Nordgaard, 1912; Runnstrom&Runnstrom, 1918-1919; Deichmann, 1930; Thorson, 1946
P. *punctatus*	14	---	360-640	brooder (depressions in sole)	Antarctic; gravel, sand, clay	Ekman, 1925; Pawson, 1969b

discovered coelomic brooding in L. antillensis, a form that apparently releases the 1.0 - 1.5 mm long juveniles, having well developed dorsal plates, through the anus.

Of the approximately 85 species contained in the family Psolidae (Pawson, 1982), there now exists at least partial information on the reproductive habits of 13 species, or 15% of all psolids; approximately 70% of the 13 species listed (11% of all psolids) brood (Table IV-2). Although most species for which we have information on reproduction are brooders, this may simply be a result of the fact that external brooding on such unique forms as psolids having a hard skeleton can be easily detected by taxonomists and those examining dredge hauls. Seven of the nine brooders listed in Table IV-2 are known only by brief descriptions from early Antarctic collecting expeditions. The high percentage of brooding members in this family may be partly due to the protective advantages afforded by the armored dorsum and the flat sole, manifest in the numerous ways in which these external body parts are utilized to incubate embryos and young. Brooding the young until they are covered with a coat of imbricating calcareous plates would be a further safeguard, especially against benthic predators in the Antarctic where epibenthic forms dominate and carnivorous asteroids are abundant in some habitats (Dayton et al., 1974). At least one Antarctic psolid, Psolus antarcticus (Ludwig, 1897b, 1898), broods young under the sole until the coat of ossicles and the flat sole have formed. Even with the coelomic brooder, Lissothuria antillensis, young remain within the female and are not expelled through the anus until they have a well-developed skeleton of imbricating plates (Miller, 1983). The coat of calcareous plates laid down by the newly settled young of P. chitonoides appears to serve as an effective shield against the predatory amphipod Parapleustes pugettensis (eggs and larvae are eaten, but newly metamorphosed young are not eaten; F. S. McEuen, unpublished data).

Although not described as bearing three ciliary rings, illustrations of the larvae and early pentactula of Psolus phantapus (Thorson, 1946) and of the early pentactula of P. fabricii (Agassiz, 1864; Agassiz and Agassiz, 1865) show three ridges present posterior to the preoral lobe. I interpret these to indicate that these two species' larvae have three ciliary rings, as we have found for P. chitonoides and Psolidium bullatum. Another trend that appears to be evident from Table IV-2 is the occurrence of pelagic larvae (or broadcasters) in the larger psolids (Psolus chitonoides, P. phantapus and P. fabricii) of 7.5

- 15 cm length, although a smaller form (Psolidium bullatum , 2.5 cm) also has a pelagic doliolaria larva. However, even within the genus Psolidium, the one brooder (P. incubans) is still smaller than the broadcasting P. bullatum. Body sizes of brooding psolids (Table IV-2) are significantly smaller than body sizes of those species that broadcast their gametes ($p=0.025$, Mann-Whitney U-test). Most of the smaller species (Lissothuria antillensis, L. nutriens, Psolidium incubans, Psolus antarcticus, P. ephippifer, P. figulus, P. granulosus, P. koehleri, and P. punctatus) with body lengths of 1.5 - 6.5 cm are brooders and appear to fit the generally observed trend among most marine invertebrate taxa of smaller adult size being associated with broodcare. Large size of psolid eggs (330 - 640 um) with the attendant brooding and doliolaria larval form is also common in other echinoderm taxa (Hyman, 1955; Hendler, 1975), but no range of egg sizes characteristic for either mode of psolid development is yet discernible.

Our current knowledge of the actual functional roles of the ciliary rings of marine invertebrate larvae is limited (Chia et al., 1984). My observations on the larvae of Psolus chitonoides show that the three ciliary rings appear just before the onset of vertical swimming behavior during which time the larvae occasionally attach to the bottom and resume swimming. Larvae are still positively buoyant at settlement, indicating that descent through the water column is by active swimming using the ciliary rings. The rate measured for downward "swimming" was more than twice as fast as upward swimming; the latter rate may be more a result of passive rising than active swimming. In addition, ciliary rings may confer increased maneuverability, enabling larvae to select shaded habitats which Young and Chia (1982) have demonstrated are preferred by pentactulae and juveniles. This is further corroborated by the fact that as the larva settles and protrudes the two primary podia, the ciliary rings are quickly lost. If settlement is postponed due to lack of a suitable substratum, the larva may become negatively buoyant with resorption of the preoral lobe and increase in size and number of ossicles; in this situation, the ciliary rings would probably also function to keep the larva in suspension.

With the positive buoyancy of the settled larva, the sticky tentacles and primary podia serve to retain the metamorphosing individual on the selected substratum until more podia and ossicles are produced and the yolk material is resorbed, thereby rendering the young psolid negatively buoyant. The numerous minute microvillar strands, globules and papillae covering the tips of the primary tentacles are probably the mechanism by which tentacles adhere to hard surfaces. Similar papillae have been found on the primary tentacles of the pentactula of Cucumaria elongata by Chia and Buchanan (1969) who found also that these papillae contain acid mucopolysaccharides that may be involved in feeding. Cilia interspersed among the hyaline papillae and the micropapillae on the hyaline papillae as well as cilia on the tube feet, presumably function for mechanoreception or chemoreception in substratum selection and for feeding in the young juvenile. Cilia and papillae are also present on adult tentacles and have been proposed by Fankboner (1978) to serve as mechanoreceptors and to entrap food particles, respectively.

The failure of settled larvae of Psolidium bullatum to complete metamorphosis in laboratory cultures could be due to one or more factors, including 1) lack of a specific settling cue, 2) a long period required for metamorphosis, or 3) the need for settled young to feed to obtain additional nutrition for completion of metamorphosis. In light of our knowledge of settlement and metamorphosis of Psolus chitonoides, the first alternative seems the most probable. Psolidium bullatum is also abundant on detritus covered rocks in quiet inlets and fjords (P. Lambert, personal communication). Although not used in my study, the milieu of detritus and silt on the tops of rocks in the adult habitat may be the stimulus for metamorphosis and an important food source for juveniles.

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V. COMPARATIVE STUDY OF THE DEVELOPMENT OF SEVEN SPECIES OF DENDROCHIROTID HOLOTHUROIDS FROM THE SAN JUAN ARCHIPELAGO, WASHINGTON, AND DEVELOPMENTAL PATTERNS IN THE ORDER DENDROCHIROTIDA (ECHINODERMATA)

A. INTRODUCTION

In areas of upwelling along the western coasts of most major land masses (North and South America, Africa, Australia) and in the Antarctic, members of the holothuroid order Dendrochirotida are commonly found in shallow subtidal marine habitats (Clark, 1931-1934, 1946; Pawson, 1963, 1964, 1969a,b, 1970; Branch and Branch, 1981). Although these suspension-feeding sea cucumbers are generally considered cosmopolitan and can be numerous at some locales in the tropics (Bennett, 1971; Endean, 1982), it is in higher latitudes in temperate or subtropical regions where they are most abundant and where species diversity is highest (Bakus, 1973).

Notwithstanding the prevalence of dendrochirotids in benthic marine communities, relatively few detailed accounts of their reproduction and development. This particular taxon of sea cucumbers is noted among echinoderms for brooding and lecithotrophic larvae (reviewed in Bronn, 1889-1892; Clark, 1902b; Ludwig, 1904; Vaney, 1925; Cuenot, 1948; Hyman, 1955; Boolootian, 1966; Inaba, 1968; Bakus, 1973). The best descriptive studies we have of dendrochirotid development through metamorphosis are for *Cucumaria echinata* (Ohshima, 1918, 1921) and *Leptopentacta* (*Cucumaria*) *elongata* (Chia and Buchanan, 1969) that possess lecithotrophic larvae. The most in-depth investigations on brooding species (excluding the family Psolidae; see chapter four) are for *C. lubrica* (Engstrom, 1982), *C. pseudocurata* (Smith, 1962; Rutherford, 1973) and *Pseudopsolus macquariensis* (Simpson, 1982). All other reports are short descriptions or brief mentions of various aspects of reproduction and development.

This chapter examines the development and metamorphosis of seven dendrochirotid species living in the plankton-rich waters surrounding the San Juan Archipelago, Washington. Five of these sea cucumbers (*Pentamera populifera*, *Eupentacta quinquesemita*, *Cucumaria miniata*, *C. piperata*, and an unidentified species of *Cucumaria*) dwell on either rock or mud bottoms (Shelford and Towler, 1925; Shelford et al., 1935, Kozloff, 1983; personal observations) and produce a pelagic lecithotrophic larva, while two species (C. lubrica, C. pseudocurata) brood (see references in Table V-3) and live on top of subtidal rocks and in intertidal beds of Mytilus californianus, respectively.

The seasonality of dendrochirotid spawning in shallow temperate habitats is marked by the appearance of vast numbers of eggs and larvae, sometimes giving a red tint to the water (Mortensen, 1927; Lacalli, 1981). In the San Juan Islands, pulses of substantial quantities of large colorful eggs are spawned by the dense beds of subtidal dendrochirotids. These eggs float on the water's surface March through May and were described by early workers at Friday Harbor Laboratories as occurring in windrows (Johnson, 1931; Johnson, 1932; Johnson and Johnson, 1950). These investigators (Johnson, 1931; Johnson and Johnson, 1950) followed the development of early holothuroid larval stages selected from the plankton and ascribed larvae to particular species based on larval color, color of ripe gonads of adults of some species, and according to an estimation of which species were locally most abundant. These criteria, however, are not sufficient for separating the numerous holothuroid larval types because 1) there are at least 11 dendrochirotid species present in large numbers in the San Juan Islands (personal observations), 2) color of ripe gonads is not a reliable indicator of egg color, and 3) larvae of many species have similar color, form, and ciliation patterns. This study separates the larval types of five dendrochirotids cultured from laboratory spawnings and provides additional details to what is already known on brooding for two other species. Information on development in this large order is assembled, excepting the Psolidae, for a clearer delineation of patterns of development.

B. MATERIALS AND METHODS

Ripe adults of each species were collected at the beginning of the different breeding seasons (see chapter two) and maintained in seawater tanks with natural substrates at Friday Harbor Laboratories. Subtidal collections using SCUBA were made at the following sites for these species: Cowlitz Bay (Waldron Island) for Pentamera populifera, Bell Island and Friday Harbor town docks for Eupentacta quinquesemita, Turn Island and Point George (Shaw Island) for Cucumaria lubrica, and Bell Island and Point George for C. miniata, C. piperata, and Cucumaria sp. Cucumaria pseudocurata was found intertidally among beds of *Mytilus californianus* at Eagle Point and Grandmother's Cove (San Juan Island) (48° 26 - 43′ N. and 122° 50′ - 123° 10′ W.).

Sexes could be distinguished in ripe *Pentamera populifera* by pressing on the ventral side or trivium (the three ambulacra with longer tube feet), which pushes the cream colored testes or green ovaries up against the bivium and makes the gonads visible through the thin body wall. In female *Cucumaria pseudocurata*, the bright orange eggs were occasionally visible through the semi-transparent body wall in the introvert region. Large males of *C. miniata* can often be identified by the genital papilla, which divides into finger-like branches, unlike the simple papilla of the female.

Gametes for culture were obtained within 1 - 2 weeks after collection from natural spawnings in the laboratory. Spawning could be induced in *Pentamera populifera* in seawater tables by turning off seawater circulation for 10 - 30 min and then resuming seawater flow with a slow current passing over the 2-liter dish of mud containing ripe adults. One to three males would usually begin spawning 10 - 20 min after water circulation was stopped, followed by numerous individuals of both sexes stretching their anterior ends out of the mud and releasing gametes when water flow was turned on. Ripe individuals of *Eupentacta quinquesemita* placed in bowls of seawater released gametes during evening and early night hours when warmed to 16 - 19° C (see chapter two). Ripe *Cucumaria piperata* also spawned after warming, but *C. miniata* and *Cucumaria* sp. did not respond to these artificial methods of spawning induction. Egg diameters were calculated from measurements made of 10 eggs from each of 3 - 7 females of each species. Eggs were obtained from spontaneous laboratory spawnings, during the breeding seasons (chapter two), with the exceptions of one female *Eupentacta quinquesemita* and two female *Cucumaria* sp., from which eggs were stripped from ripe ovaries.

Procedures for fertilization, culturing, and examination of developmental stages using scanning electron microscopy were the same as those described in chapter three.

C. RESULTS

GAMETES

A listing of sizes and colors of the gametes is given in Table V-1. All species' eggs are relatively large, with maximum sizes found in the two brooders (Cucumaria lubrica and C. pseudocurata). Of the five dendrochirotids producing pelagic larvae, three species (C. miniata, C. piperata, and Cucumaria sp.) positively buoyant eggs (C. miniata, C. piperata, and Cucumaria sp.) and two (Pentamera populifera and Eupentacta quinquesemita) have eggs that are slightly negatively bouyant; eggs of the two brooders are very negatively buoyant (chapter two). The elongate sperm morphology of the two brooding species departs from the usual primitive type of sperm common to echinoderms (Atwood and Chia, 1974; Atwood, 1975a,b).

DEVELOPMENT

With the exception of the two brooding species with larger eggs, embryonic development in the other five dendrochirotids is characterized by equal, holoblastic cleavage resulting in a ciliated coeloblastula which hatches and develops into a pelagic lecithotrophic larva. The timetables of developmental events for the various species are contained in Table V-2. Due to the similarity of embryonic and larval development of Cucumaria miniata, C. piperata, and Cucumaria sp. to Pentamera populifera, the three species are grouped under one subheading and only major differences in their larval morphology and behavior are noted.

Pentamera populifera

After gastrulation (at 30 hr), the light green embryo lengthens into a uniformly ciliated larva (44 hr) (Fig. V-1A) and commences vertical swimming behavior, rotating counter clockwise (viewed from apical end). Three faint epidermal ridges are noticeable on the posterior half of the early doliolaria (Fig. V-1B) at 2 1/4 d; they are more prominent at 2 1/2 - 3 d to give the appearance of there being three ciliary rings. With the up-and-down swimming, occasional larvae begin to swim at 2 1/2 - 3 d along the bottom of the culture dish in a horizontal position. The 3 3/4 d-old doliolaria (Fig. V-1C) has

Table V-1. Characteristics of gametes from seven species of dendrochirotid holothuroids. All sperm measurements, with the exceptions of *Pentamera populifera* and *Cucumaria* sp., are taken from Chia et al. (1975) and Fontaine and Lambert (1976). Egg colors are taken from chapter two. Values for egg sizes are means ± 1 standard error; numbers in parentheses under egg diameter indicate number of females for which diameters of 10 eggs each were measured.

SPECIES	SPERM LENGTH (um), SHAPE	EGG DIAMETER (um)	EGG COLOR
Pentamera populifera	4.6 (spherical)	372 ± 5 (3)	light green
Eupentacta quinquesemita	4.0 (spherical)	397 ± 1 (6)	light green
Cucumaria miniata	3.5 (spherical)	520 ± 3 (5)	green
C. piperata	4.0 (spherical)	532 ± 3 (5)	olive green
Cucumaria sp.	--- (spherical)	504 ± 3 (6)	tan
C. lubrica	7.0 (cylindrical)	973 ± 3 (7)	dull yellow
C. pseudocurata	5.5 (tabloid)	1051 ± 9 (5)	bright orange

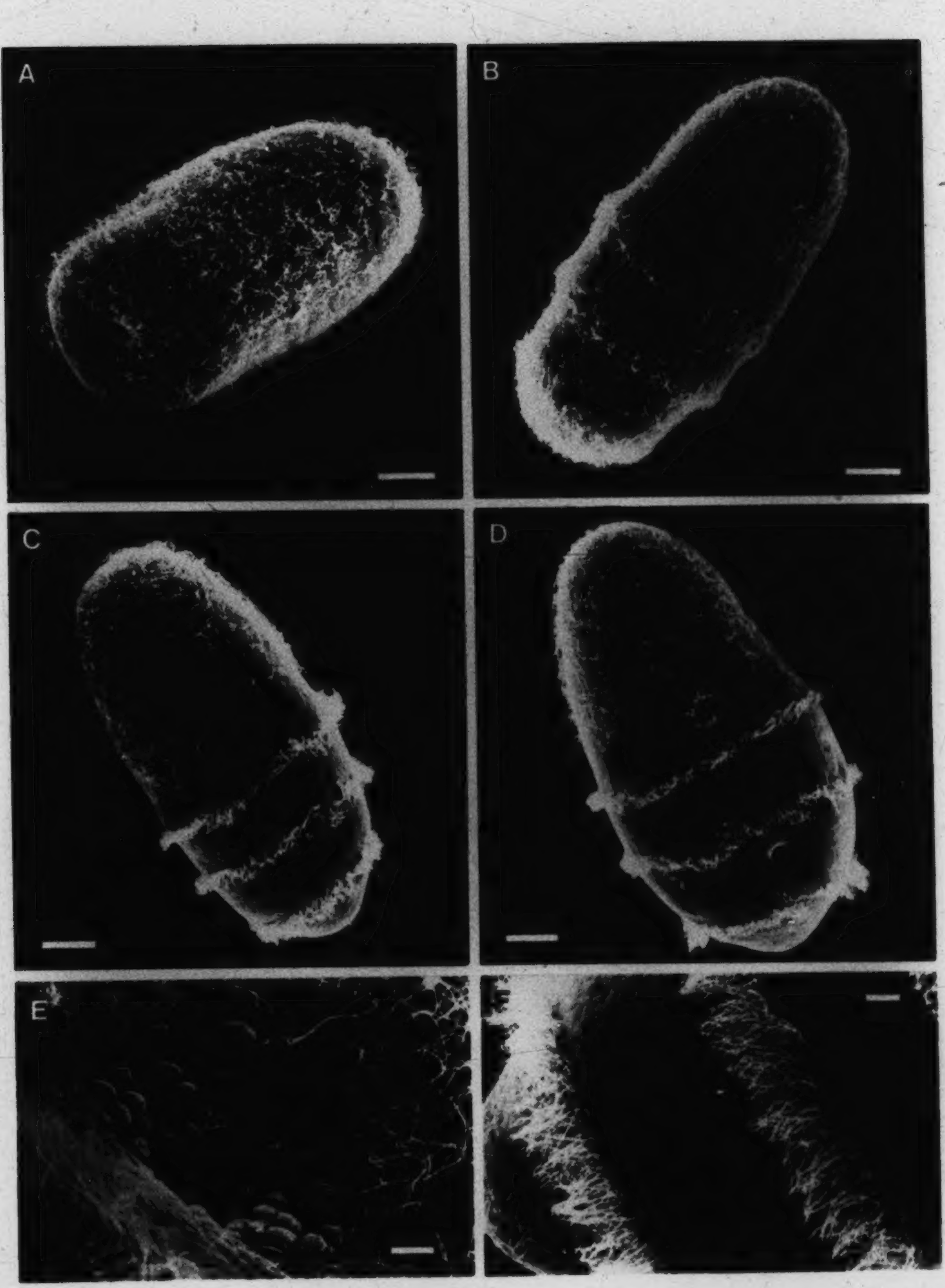
Table V-2. Development schedule for seven species of dendrochirotid holothuroids raised at 11° ± 0.5° C at Friday Harbor Laboratories. Variability in development rate increases with age and is indicated by number of significant figures used with recorded times of developmental stages. Numbers in parentheses are ranges of larval or juvenile sizes in um. Dashes indicate no data, na (not applicable) signifies the stage is not found in that species, and "early" means early blastula. Pp = Pentamera populifera, Eq = Eupentacta quinquesemita, Cm = Cucumaria miniata, Cp = C. piperata, Csp = Cucumaria sp., Cl = C. lubrica, Cps = C. pseudocurata.

Stage	Pp	Eq	Cm	Cp	Csp	Cl	Cps
fertilization to 2-cell	3.0 h	5.0 h	2.8 h	2.0 h	3.0 h	9-12 h	10-16 h
4-cell	4.0	6.0	4.5	3.0	3.8	11-23	15-21
8-cell	5.3	7.0	5.5	4.0	4.5	12-26	16-28
16-cell	6.3	8.0	6.8	5.0	5.3	14-33	23-30
32-cell	7.3	9.5	9.0	6.8	6.5	20-38	29-37
64-cell	8.5	11.0	11.0	8.8	8.0	25-41	30-42
blastula	14.5 (early)	18.0	16.8 (early)	13.8 (early)	--	--	--
hatching	23	28	33	32	--	32-35 d (975-1190)	36-37 d (945-1250)
gastrula	30	33	41	41	--	3 1/2 - 6 1/2	7 1/2 - 9 1/2
early larva (uniformly ciliated)	44 (540-630)	51 (555-660)	57 (600-885)	62 (620-815)	62 (625-670)	na	na
early doliolaria (with epidermal ridges)	2 1/4 d (470-550)	2 3/4 d (510-570)	3 1/2 d (675-765)	3 1/2 d (680-740)	3 3/4 d (675-725)	--	--
primary tentacles (first visible)	5 3/4	4 1/4	5 3/4	7	7 1/2	at hatching	at hatching

primary podia	10 1/2 d	6 d	7 d	8 d	8 d	34-38 d	at hatching
settlement	6-7 1/2 (490-600)	6 1/2-7 1/2 (495-675)	8-13 (700-800)	8-9 1/2 (740-770)	9 1/2-10 3/4 (600-710)	na	na
first ossicles	4 3/4	7 3/4	10 1/4	10	11 1/2	34-37	40-41
"armored" juvenile	9-15 (545-630)	11-16 1/2 (540-700)	29-30 (875-1230)	22-25 (925-1325)	18-21 (700-1125)	na	na
earliest feeding	17	23	--	--	--	*10-14 wk	-

*from Engstrom, 1982

Fig. V-1. _Pentamera_ _populifera_: (A) Early larva with uniform ciliation. (B) Early doliolaria larva. Epidermal ridges forming and cilia becoming more dense at future sites of ciliary rings. (C) Dorsal view of doliolaria with three transverse ciliary rings and an evenly ciliated preoral lobe. (D) Vestibule (at posterior border of evenly ciliated zone) and two podial pits (between second and third ciliary rings) on the venter. Scale bars for Figures V-1A through D = 50 um. (E) Close-up of vestibule of doliolaria. Scale bar = 10 um. (F) Dorsal hydropore (arrow) posterior to the second ciliary ring.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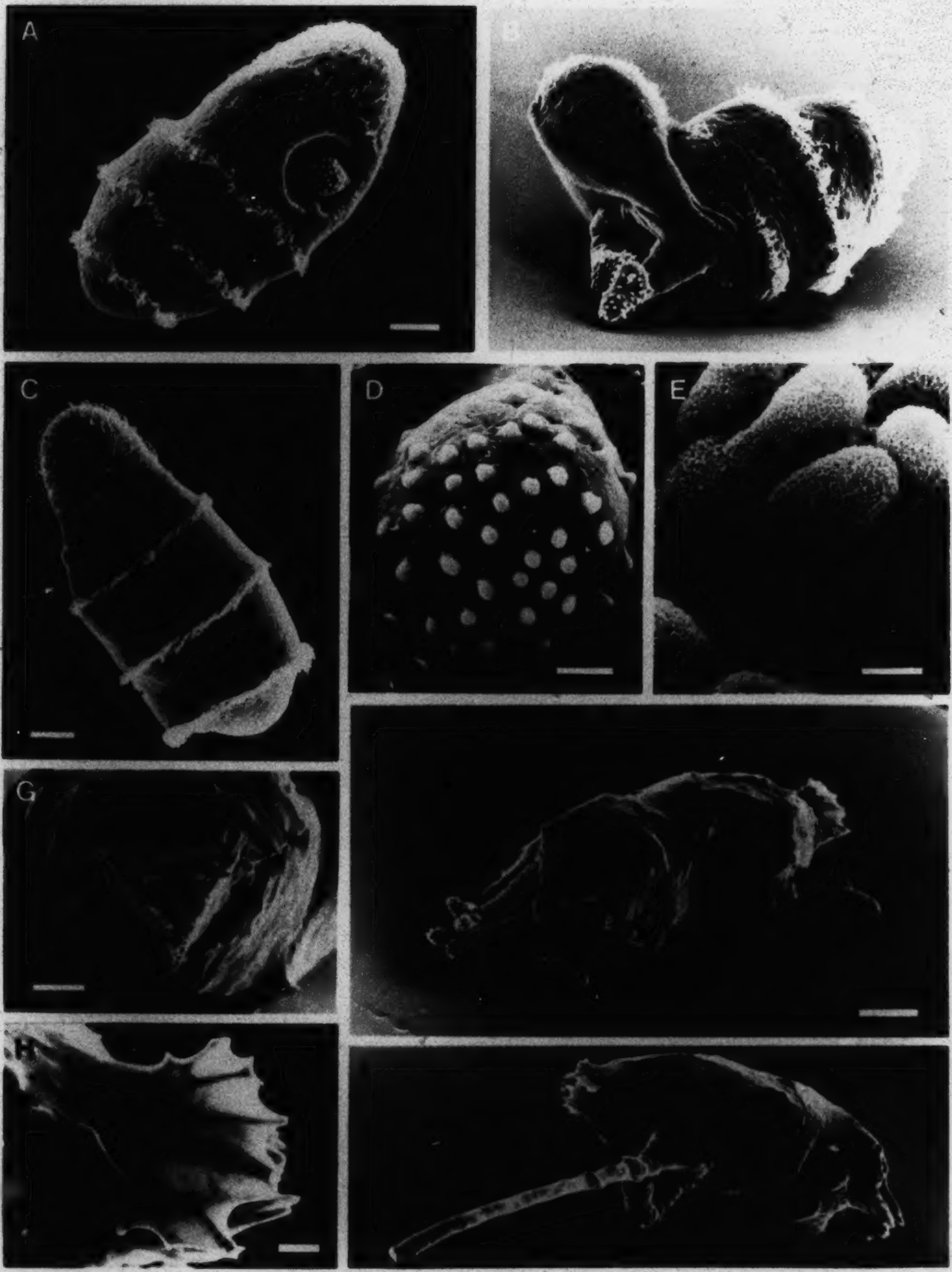
well-defined ciliary rings and is demersal.

At 5 d and 7 hr after fertilization, five extensions of the hydrocoel (rudiments of the five primary tentacles) are visible within the doliolaria larva. The doliolaria now possesses two regions of different coloration: an anterior yolky portion (35 - 40 % of the length of the larva) pigmented light green and a light white, semi-opaque posterior. Fourteen hours later, the vestibule can be seen at the border where the green and white areas meet (Fig. V-1D, E) and the hydropore is visible posterior to the second ring of cilia (Fig. V-1F). The ossicles first show posterior to the third ciliary ring at 4 3/4 d. Larvae cultured at room temperature (16 - 19° C) swam backwards on the dish bottom with the posterior end leading; doliolaria larvae incubated at 11° C also swam on the bottom of the dishes, but with the preoral lobe forward. A 5 3/4 d-old larva is 465 - 510 um long with a flattened ventral surface in the area of the vestibule. It is at this age that the first larvae are seen with primary tentacles sticking out of the vestibule (Fig. V-2A). Emergence of the tentacles pushes the preoral lobe to an angled position with the main axis of the larval body (Fig. V-2B). Dorsal to the tentacles and perpendicular to the main body axis, a clear line marks the beginnings of the calcareous ring (at 6 - 6 1/4 d). From the time of the early doliolaria, the preoral lobe undergoes a gradual diminution in size, giving the lobe a rounded conical shape with a diameter smaller than the posterior portion supporting the ciliary rings (Fig. V-2C).

Behavior of the doliolaria larva up to time of settlement consists of spinning rapidly parallel to and along the dish bottom and occasional swimming to the water surface; the latter is always in a vertical position with the preoral lobe uppermost. The larvae also aggregated in small clumps on the bottoms of the culture dishes as has been described for Psolus chitonoides (Young and Chia, 1982). Doliolaria larvae in cultures kept in seawater tables subject to bright sunlight entering from south facing laboratory windows gathered in dense aggregations towards the light. This latter behavior also continued throughout settlement. Settlement occurs at 6 - 7 1/2 d post-fertilization and attachment to the substratum is with the primary tentacles (Fig. V-2B). Figures V-2D,E Recently settled pentactulae, while the buds of the two primary podia are emerging, lie on their sides or on the dorsum. The preoral lobe is small when settlement takes place and is completely resorbed in the following 1 - 2 d. Bifurcation of the tentacles also starts within the first 2

Fig. V-2. Pentamera populifera: (A) Primary tentacles of doliolaria beginning to protrude from the enlarged vestibule. Scale bar = 50 um. (B) Settling pentactula. Reducing preoral lobe is pushed back to a more dorsal location as tentacles assume a more terminal position. (C) View of left side of doliolaria with reducing preoral lobe. Bulge (arrow) is due to tentacles withdrawn into vestibule. Scale bar = 50 um. (D) Papillae on newly formed tentacle of doliolaria. Scale bar = 10 um. (E) Tentacle papillae of recently settled young. Scale bar = 2.5 um. (F) "Armored" pentactula with covering of new ossicle plates and two primary podia. Five plates surround and interdigitate with the five primary tentacles. Scale bar = 100 um. (G) End view of three plates surrounding anus of armored pentactula. Scale bar = 40 um. (H) Growth pattern of anal ossicle plate evident through thin epidermis (lateral view). Scale bar = 20 um. (I) Elongate primary podia, characteristic of recently settled pentactulae of this species.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d after settlement. Ossicles form a calcareous armor over most of the body trunk at 9 - 15 d (Fig. V-2F).

The actual time of first ingestion of food is uncertain since 1) the primary tentacles are alternately stuffing themselves into the vestibular cavity as soon as they emerge and 2) the time of formation of the buccal cavity and gut are not known. Larvae provided algae (Dunaliella tertiolecta and Isochrysis galbana) at settlement, however, are defecating boluses at 17 d. The simple, looped gut at this time consists of the pharynx, stomach, and intestine and can be easily distinguished using substage illumination. With the further proliferation and growth of the body wall ossicles, metamorphosis can be considered complete when the main body region becomes covered with a coat of calcareous mail. The five large anteriormost ossicles encircle the buccal region (Fig. V-2F) and interdigitate with the five primary tentacles. Three plates surround the anus (Fig. V-2G, H). Tentacle growth occurs at the tip of the tentacle; the three dorsal tentacles now have two primary branches behind the main leading branch and the two ventral tentacles have only a simple bifurcation. By 18 d, the two primary podia that appeared 2 - 3 d after settlement can stretch up to 2.5 times the juvenile body length (Fig. V-2I). Small ossicles encircle the length of the primary podia in a spiral fashion with only glints of new ossicles evident in the tentacles.

The bud of the third primary branch on the dorsal tentacles is visible at 31 d and the fourth and fifth primary branches are present by 36 d. Secondary branches have also begun to show on the first two primary branches; ossicles, as well, are present at the bases of the latter. The two ventral tentacles yet remain with simple forked ends at the end of 62 d and only a sixth primary tentacle branch has been added to the three dorsal tentacles. No additional podia have appeared.

Juveniles collected from Cowlitz Bay in the last week of April, 1983 (approximately 6 - 8 wk old), had a mean length of 1038 ± 173 um (s.d., n=5) and fed in laboratory bowls by either suspension or deposit feeding. These young were also with a full coat of ossicles and two primary tube feet; the five primary tentacles supported 5 - 7 primary tentacle branches.

Eupentacta quinquesemita

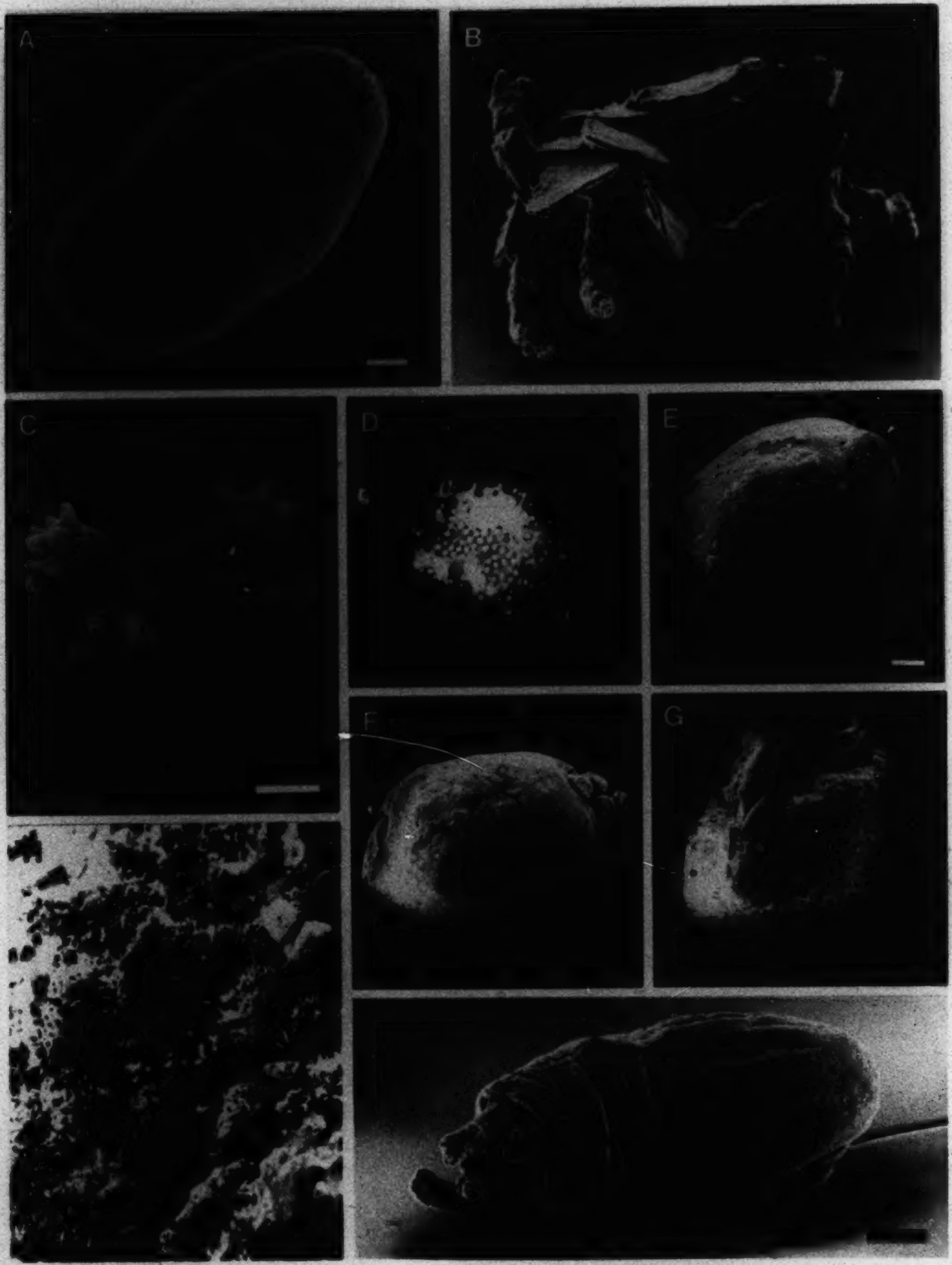
The 18-hr blastula of Eupentacta quinquesemita is wrinkled and is divided into quarters by four main egression tracts or wrinkles. Blastulae begin to smooth out and become more spherical in shape from 23 - 26 hr. The blastular wall at the vegetal pole then increases in width and length which gives this area a whitish opaque color along the periphery. After hatching, the embryo measures 445 - 460 um long, rotates in a counter clockwise direction and continues to elongate; the vegetal plate becomes evident by 30 hr and gastrulation commences 3 hr later.

Further lengthening after the gastrula stage results in a uniformly ciliated larva; this even ciliation of the larva is retained throughout the larva's pelagic life (Fig. V-3A). By 48 hr, the larva has reversed its direction of spinning. Five hours later, larvae at the water surface swim with the apical end forward while those on the dish bottom swim in reverse with the blastopore end leading. The vestibular pit opens at 2 1/2 d, accompanied by a dorso-ventral flattening of the larval body. Podial pits open up at 3 1/4 d and 4 d, the first in the posterior ventral portion of the larva directly below the vestibule (Fig. V-3A) and the second on the right side of the larva at the same level and at a 90° angle to the first. Length of the larva ranges 530 - 650 um. This larva is similar to that of Pentamera populifera in having the anterior yolky preoral lobe pigmented light green and a semi-translucent white posterior.

Tentacles begin emerging from the vestibule at 4 1/4 d and are widely splayed out during swimming; Thorson (1946) referred to the pelagic pentactula as the "swimming-crawling" stage. Toward the end of the pelagic period, the size of the light lime green preoral lobe is greatly reduced and larvae put out the first two tube feet just prior to or during settlement. Larvae settle at 6 1/2 - 7 1/2 d, attaching to the bottom and sides of the bowl. No irregular distributions of settled larvae were obvious in bowls subject to bright sunlight from windows on the south side of the laboratory.

Resorption of the remaining small preoral lobe is complete 1 - 1 1/2 d after settlement, resulting in a whitish semi-opaque pentactula. Pentactulae become covered with large ossicles at 11 - 16 d and measure 540 - 700 um (Fig. V-3B). Bifurcation of the tentacle tips is noticeable by 16 - 17 d, along with the first secondary podium that is directly anterior to the left primary podium. Another secondary podium appears (at 30 d)

Fig. V-3. Eupentacta quinquesemita: (A) Uniformly ciliated doliolaria. Scale bar = 50 um. (B) Heavily armored pentactula. Scale bar = 100 um. Cucumaria sp.: (C) Tentacle growth in young juvenile. Arrows indicate separation of papillae that will cover the ends of the tertiary tentacle branches. Scale bar = 20 um. C. lubrica: (D) Ventral view (through plexiglass wall of seawater table) of a female brooding early embryos; tentacles remain retracted during brooding. Magnification = 1.75x. (E) Pentactula commences hatching by pushing primary tentacle (arrow) through egg membrane. Scale bar = 100 um. (F) Recent hatchling with five primary tentacles. Arrow points to one primary podium just beginning to form. (G) Torn, tough vitelline membrane left behind after hatching. (H) Field photograph at Turn Island of white, yolky young (arrows) that are coming out from underneath two females. 12 - 15 m depth. Magnification: 1.3x. (I) Three- to four-day old pentactula. Scale bar = 200 um.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in front of the right primary podium, but in a staggered position behind the other secondary tube foot. The primary podia, now well endowed with ossicles, are capable of stretching the length of the pentactula and have been active in adhering to and walking on the substratum since their emergence from the podial pits. At a size of 950 - 1175 um and 23 d old, fecal boluses are first produced. The three dorsal tentacles each support three primary branches at this time and add a fourth branch at 33 d.

Cucumaria miniata, C. piperata, and Cucumaria sp.

The gastrula of Cucumaria miniata is a rounded cone in outline that lengthens into a bullet-shaped early doliolaria. The three-ringed doliolaria in this species is a sluggish swimmer and is more rectangular and robust in form than other larvae examined in this study. The vestibule can either be wide and cavernous or only a slight depression, but is usually the latter, so that the tentacles come straight out and not from within a cavity. Ossicle formation commences in the posterior third of the larva, the ossicles that will later encircle the anus being the most prominent. Larval pigmentation differs from the green egg: the preoral hood is olive green and the remaining two-thirds of the larva is light green. The sequence of tube foot development follows the normal pattern for dendrochirotids as detailed by Ohshima (1921): two large primary tube feet emerge from podial pits followed by small, slender secondary tube feet. Of the latter, the first is located in front of the left primary tube foot and the second in front of the right primary tube foot and anterior to the first secondary tube foot.

In Cucumaria piperata, the gastrula does not have the pronounced cone shape as in C. miniata. Typical of all three cucumariids, a very noticeable indentation is present on the right side of the early doliolaria, between the second and third ciliary ridges. This depression is the site of one of the podial pits which gradually assumes a ventral position alongside the other podial pit by the time the tube feet appear at or just before settlement. This doliolaria with olive green preoral lobe, light green body, and three ciliary rings is difficult to tell apart from the C. miniata doliolaria. The preoral lobe in the settling C. piperata pentactula is yellowish green and later fades to a light yellow as it is resorbed. The five rows of imbricating ossicles in the "armored" juvenile are quite uniform in this species, like those described by Chia and Buchanan (1969) for Leptopentacta (=Cucumaria) elongata.

Although resembling larvae of Cucumaria miniata and C. piperata in form and size, the early Cucumaria sp. doliolaria larva can be distinguished by the orange preoral lobe and light yellow posterior. The pigmentation of the larva separates into two clearly distinct regions of orange and yellow by day five (although larvae from one female were bright yellow). The two anterior ciliary rings are first to become distinct, followed by the third ciliary ring as cilia on the posterior end disappear. The early larval stage of Cucumaria sp. is also marked by a cleft arising from the site of the former blastopore which has since closed over. The cleft divides the posterior 15 - 20 % of the larva into two lobes that disappear in the later doliolaria just before the swimming period ends. Recently metamorphosed young are a light orangish yellow and retain a yolky appearance just after resorption of the preoral lobe.

Examination of the larvae of these three species using electron microscopy revealed that the prominent ciliary ridges on the early doliolaria give the illusion of three transverse rings of cilia. The ciliary rings, however, are not distinct until cilia between the ciliary ridges are shed (resorbed?) from the epidermis just prior to the up-and-down swimming phase (as noted for Psolus chitonoides, chapter four). A general observation on behavior in cultures exposed to bright sunlight from laboratory windows was the formation of dense aggregations on the sides of the bowls away from the light source, which has also been observed for P. chitonoides (Young and Chia, 1982; chapter four).

Cucumaria lubrica

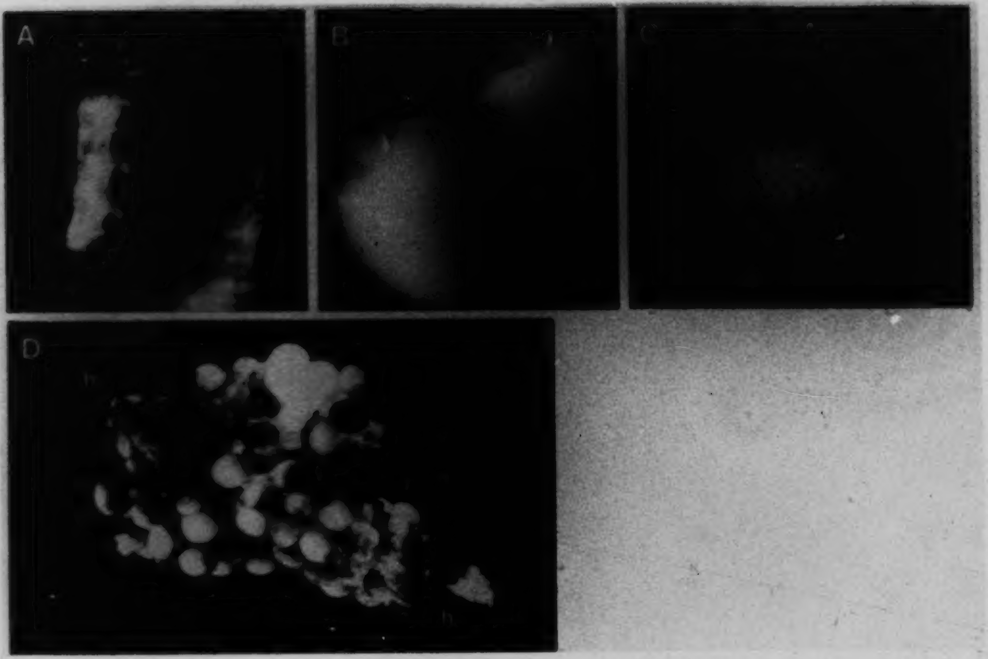
Development is direct in this brooding dendrochirotid (Atwood and Chia, 1974; Engstrom, 1974, 1982) and takes place within a clear egg membrane underneath the trivium of the female (Fig. V-3D). Small depressions show up on the egg surface under the membrane after fertilization, but vanish before the first cleavage. The first 2 - 4 cleavages can be equal but are mostly unequal; cleavage is superficial and becomes irregular and out of synchrony within a clutch as early as the 8- and 16-cell stages. The onset of gastrulation is hard to discern externally with the cleavage irregularities and egg opacity. At 4 - 4 1/2 d, small dimples and small crevices are visible on the embryo surface.

A "pre-hatching" stage (appearing 18 - 22 d) is characterized by faint outlines of the ends of the primary podia immediately below the egg membrane. Hatching begins in the tentacular region with one tentacle pushing through the membrane (Fig. V-3E), leaving behind the torn egg membrane (Fig. V-3G). Hatching is drawn out for a period of 1/2 - 1 1/2 d. Figure V-3F shows the new hatchling with five tentacles. The hatchlings rapidly increase in length (up to 2 mm) and grow two short podia (Fig. V-3I) within 2 - 3 d post-hatching. Engstrom's (1982) reported longer hatching time of 41 - 45 d is likely due to colder seawater temperatures (7 - 8° C, December through January) in his laboratory tanks. He estimates a post-hatching brooding period of 4 - 8 wk before juveniles crawl out from under the females. On 21 January, 1982, I examined 115 broods at Turn Island and found two with juveniles up to 2 cm away from the female and four females where the young were just beginning to come out. A year later on 10 February, 19 % of the female population (19 of 97) were releasing brood (Fig. V-3H), while 8 % of the females (11 of 140) at Point George on 15 February had juveniles crawling out. An extensive search of the population on the vertical wall at the Point George site on 25 February, 1984, turned up only three broods being released.

Cucumaria pseudocurata

External brooding with direct development has been mentioned or briefly described for Cucumaria pseudocurata by Smith (1962), Rutherford (1973), and Turner and Rutherford (1976 a,b). Eggs are enclosed in a tough, cellophane-like membrane and are sticky, adhering together as a mass after being spawned. Tube feet of the female trivium cling tenaciously to the eggs and to the substratum (Fig. V-4C). Smith (1962) noted cleavage to be superficial. Occasional fertilized eggs pinched off a large globule of cytoplasm (Fig. V-4B). My observations on cleavage showed it to lack any kind of order or regularity for a single embryo or among embryos of the same brood. The pre-hatching stage is reached at 19 - 24 d and includes rudiments of the two primary podia. The young hatch as pentactulae (Rutherford, 1973; Turner and Rutherford, 1976 a, b) with two tube feet. Hatchlings attach immediately to the substrate with tentacles and podia after leaving the tough egg membrane (Fig. V-4D). Throughout development there is a slow change of color from a bright orange in the early cleavage stages to a dull yellow toward the close of

Fig. V-4. Cucumaria lubrica: (A) 2 1/2 month old juveniles, 3.5 mm in length. C. pseudocurata: (B) Fertilized eggs with pinched off cytoplasm. (C) View of venter of brooding female. Magnification: 2.5x. (D) Brood mass of embryos and hatching young. Different developmental stages present, from unpigmented late embryonic stages to pigmented recent hatchlings (h). Numerous pre-hatching stages are identifiable by the faint circles that indicate primary tentacles and tube feet. e is for egg membrane. Arrow points to hatching individual. Magnification: 7x.


A
B
C
D

the embryonic period and then to a dark brown in the hatching young. Four additional podia are added in the 4 d following hatching, two at a time and one each in front of the earlier pair of podia. The tentacles at this time start to bud branches and the first ossicles are barely visible.

At Eagle Point on 28 January, 1983, 13 % of the embryos of 11 broods had hatched; four of 67 adults on 22 February were releasing young. Nematodes and syllid polychaetes were found among some broods, but no evidence of predation or other adverse effects were noted.

For both brooding species, embryos separated from the female at spawning and placed in separate bowls followed a normal course of development. Though Smith (1962) did not state the temperature at which he raised his broods, his development schedule for Cucumaria pseudocurata was quite rapid (tentacles and mouth formed at 4 d, podia at 5 d, and young with 10 tentacles dispersing in the second wk).

FIELD SETTLEMENT SITES

During the first week of July, 1982, a heavy settlement of Pentamera populifera was encountered among conspecific adults in Cowlitz Bay: the settled 1 - 2 mm juveniles either rested on top of the mud or were in the top 1 - 2 mm of sediment with tentacles extended above the mud surface. The young sea cucumbers formed a fine white fuzz over the mud; estimated density of these new recruits was 1.5 x $10^5/m^2$, based on field photographs (photographs courtesy R. Shimek). For Eupentacta quinquesemita, young of the year are common May to August attached to hydroids, algae, and sabellid tubes on tops of large boulders and on rock walls in high current channels and on docks and pilings. At Crane Island on 6 July, 1983, newly settled pentactulae of Cucumaria miniata and C. piperata were discovered adhering to the undersides of rocks; 12 1 - 2 mm recruits were counted on the bottom of one rock with an area of approximately 525 cm^2.

D. DISCUSSION

BROODING

From the summary of information on dendrochirotid development in Table V-3 and from the results of the present study, Mortensen's (1920) statement that all eggs of dendrochirotids are large and yolky still holds true. In addition to those cases of brooding reviewed by Boolootian (1966), two more brooding species not included by him along with seven other new reports since his review are listed in Table V-3. Of the 39 species for which development is known (Table V-3), 24 (62 %) brood and 15 (38 %) free-spawn their eggs that develop in the plankton. A break-down of the different brooding habits can be made to illustrate the diversity of methods employed to protect developing young:

a) 2 - 3 dorsal pouches positioned in the interambulacra behind the tentacles (Cherbonnier, 1972, reported a single dorsal pouch for Neocnus incubans);

b) two ventral, interradial pouches also located behind the tentacles;

c) pouches in each of the five interambulacra just posterior to and encircling the tentacles;

d) in ventral pockets or depressions of the sole;

e) underneath the trivium against the substratum (rock);

f) in the coelom, and;

e) for a brief period among the tentacles.

Thus far, only direct development has been indicated in these brood-protecting forms, except for two cases: 1) in Phyllophorus urna where uniformly ciliated larvae swim in the coelom, and 2) in Ocnus planci that retains eggs among the tentacles for a short time that later develop into doliolaria larvae with four ciliary rings. These two exceptions come from the old literature and have not been studied in detail. It would be interesting to know if P. urna is larviparous and if the supposed tentacle brooding for O. planci truly is as it has been described or if it was a coincidental occurrence of eggs being caught on the tentacles or falling onto the buccal membrane after spawning.

Brooding dendrochirotids can also be placed into the two broad groupings of those that use coelomic incubation and those that brood in pouches, depressions in the sole, or underneath the trivium. When categorized as such, it is seen that four of the five

Table V-3. Tabulation of adult size, egg size, habitat and distribution for species of the holothuroid order Dendrochirotida (excluding the family Psolidae) with known modes of development. Dashes indicate no data available.

Species	Maximum Adult Length (cm)	Egg Size (um)	Developmental Mode	Habitat, Distribution	Sources
Aslia lefevrei	15	400-650	uniformly ciliated larva	in crevices, under stones; Orkneys to Mediterranean	Orton, 1914; Newth, 1916; Olaso Toca, 1982; Costelloe &Keegan, 1984a,b; Costelloe (in press), personal communication
Cladodactyla crocea	10	600-700	brooder (2 dorsal pouches)	*Macrocystis* fronds &holdfasts, rocks, ascidians; subantarctic, Antarctic	Thomson, 1878a,b; Ludwig, 1887, 1898b; MacBride&Simpson, 1908; Feral&Doumenc, 1982
Cucumaria coatsi	5.5	---	brooder (ventral interradial pouches)	Antarctic	Ekman, 1925
C. echinata	10	400-440	doliolaria (3-4 ciliary rings)	sand, shell; Japan	Ohshima, 1918, 1921
C. frondosa	30-60	460-670	uniformly ciliated larva	on, under rocks; Greenland to Nantucket, Iceland, Norway, England	Danielssen&Koren, 1856; Agassiz&Agassiz, 1865; Whiteaves, 1901; Clark, 1901a, 1902b; des Arts, 1910; Nordgaard, 1912; Runnstrom&Runnstrom, 1918-1919; Mortensen &Lieberkind, 1928; Thorson, 1946; Jordan, 1972; Lonning, 1976; Falk-Petersen, 1982a,b,

(*C. frondosa*)			young swim in coelom near anus		Fabricius, 1780(in Edwards, 1910)
" "			red auricularia with 4-5 bands of cilia		Ganong, 1888(in Edwards, 1910)
C. georgiana	7	1000	brooder (2 latero-dorsal interradial pouches)	Antarctic	Vaney, 1906, 1907; Ekman, 1925; Pawson, 1969b
C. ijimai	3.1	500-550	brooder (2 ventral interradial pouches)	pebbles; N.W. Pacific	Ohshima, 1915
C. joubini	4.5	1200	brooder (5 interradial pouches near tentacles)	mud, sand, gravel; Antarctic	Vaney, 1914
C. lamperti	3.4	800	brooder (2 ventral pouches)	sand, shells, gravel; N.W. Pacific	Ohshima, 1915
C. lubrica	4.5	900-1050	brooder (under trivium)	subtidal rock walls, cobble; Alaska to California	Atwood&Chia, 1974; Engstrom, 1982; this study
C. miniata	37	520-550	doliolaria (3 ciliary rings)	under rocks(subtidal, intertidal); Alaska to California	M. Strathmann, 1974 Brumbaugh, 1980; this study
C. piperata	10	532	doliolaria (3 ciliary rings)	mud, cobble, shale; British Columbia to Baja California	Deichmann, 1937; Brumbaugh, 1980; this study
C. pseudocurata	2.5	1000-1051	brooder (under trivium)	intertidal rock faces, under algae, *Mytilus* beds; Alaska to California	Clark, 1901a, b; Cowles, 1907; Brumbaugh, 1964; MacGinitie& MacGinitie, 1968; Smith, 1962; Rutherford, 1973; Turner& Rutherford, 1976a, b; Lambert (in press); this study

C. vaneyi	2.2	---	brooder (2 dorsal pouches)	Antarctic	Cherbonnier, 1949
Cucumaria sp.	23	504	doliolaria (3 ciliary rings)	subtidal cobble fields;N.W. Pacific	this study
Eupentacta quinquesemita	12	397-420	uniformly ciliated larva	on rocks,docks, pilings;British Columbia to California, Japan	Mitsukuri, 1912;M. Strathmann, 1974;Brumbaugh, 1980; Byrne, 1983;this study
Leptopentacta elongata	6.3	250-350	doliolaria (4 ciliary rings)	mud,sand;Iceland to France	Ursin, 1960;Fish, 1967;Chia &Buchanan, 1969
Neoamphicyclus lividus	4.5	370	brooder (in coelom)	under stones (littoral);N. Zealand,Tasmania, Australia	Hickman, 1978;Rowe&Vail, 1982
Neocnus incubans	0.6	200-500	brooder (dorsal pouch)	on algae(*Cystoseira*); Mediterranean, No. Africa	Cherbonnier, 1972
?*Neocnus* sp nov.	---	---	brooder	littoral;Victoria, Australia	Rowe&Vail, 1982
Ocnus glacialis	3	1000	brooder (2 ventral interradial pouches)	Arctic,No. Atlantic	Levinsen, 1886;Mortensen, 1894;Gerould, 1898;Clark, 1902a;Thorson, 1936;Cherbonnier, 1972
O. planci	15	large (less than 500 um)	doliolaria (4-5 ciliary rings;eggs brooded briefly among tentacles)	mud;North Sea to Mediterranean,W. Africa,Bahamas	Kowalevsky, 1867;Selenka, 1876a,b;Ludwig, 1891; Deichmann, 1957;Ursin, 1960;Chadwick, 1891
O. sacculus	1.7	---	brooder (3 dorsal interradial pouches)	muddy sand; N. Zealand	Pawson, 1983
Pachythyone lugubris	1.5	---	brooder	sand(shallow);	Deichmann, 1939, 1941

			(in coelom)	Lower California	
P. rubra	4	---	brooder (in coelom)	crevices,holdfasts; California	Clark,1901a,b;Deichmann, 1939;MacGinitie&MacGinitie, 1968;Chaffee,1982
Pawsonia saxicola	10	400	uniformly ciliated larva	crevices,under rocks; England,France	Orton,1914;Newth,1916; Feral&Doumenc,1982
Pentamera populifera	7	372	doliolaria (3 ciliary rings)	mud;N.W. Pacific	this study
P. pulcherrima	10	---	pelagic larva	mud,sand,*Zostera* beds;Massachusetts to Gulf of Mex., Caribbean	Clark,1902b;Grave,1905;Coe, 1912;Deichmann,1954; Miller&Pawson,1984
Phyllophorus urna	---	---	brooder (uniformly ciliated larvae swim in coelom)	Mediterranean	Kowalevsky,1867;Ludwig, 1898a
Pseudocnus laevigatus	12	1000-1500	brooder (2 ventral pouches)	sand,rock,shell; circumpolar:subantarctic,Antarctic	Lampert,1889;Gerould,1898; Ackermann,1902;Pawson,1964, 1968,1971;Simpson,1982
Pseudocnus sp. nov.	---	---	brooder	littoral;Victoria, Australia	Rowe&Vail,1982
Pseudocucumis africanus	8	---	brooder (in coelom)	under coral, rock; intertidal *Mytilus* beds;E. Africa to Rotuma	Ohshima,1916;Clark,1976 Lawrence,1979
Pseudopsolus macquariensis	2.5	1000-1800	brooder (2 ventral interradial pouches)	under rock(littoral); Kerguelen Is.	Ludwig,1898b;Mortensen, 1925;Pawson,1968;Cherbonnier&Guille,1974;Simpson, 1982
Sclerodactyla briareus	22.5	250-300	pelagic egg (direct development inside egg membrane)	soft mud,estuarine; Massachusetts to Texas	Clark,1902b;Pearse,1908; Ohshima,1925;Colwin&Colwin, 1956;Miller&Pawson,1984
Staurothyone inconspicua	1.7	---	brooder	So. Australia	Clark,1946

Stereoderma kirschbergi	---	---	uniformly ciliated larva	Mediterranean	Kowalevsky, 1867(from Ohshima, 1921)
Thyone fusus	20	350	pelagic egg (direct development within egg membrane)	shell bottom; Norway to Mediterranean	Mortensen, 1927; Costelloe, personal communication
T. imbricata	3.4	1200	brooder (2 ventral pouches)	sand, pebbles; N.W. Pacific	Ohshima, 1915
Trachythyone parva	7.5	200	brooder (depressions in sole)	Antarctic	Ludwig, 1898b; Clark, 1946

coelomic brooders are warm water inhabitants and that 15 of the 16 species in the second category (13 of them utilizing pouches) are distributed in cold water habitats (8 in the subantarctic-Antarctic, 5 in the northwest Pacific, and 1 each in the Arctic, Mediterranean, and New Zealand). Details of the ecology and development of most of these holothuroids have not been studied, but whether a species adopts coelomic or pouch brooding might be determined by stressful conditions in the habitat and mode of nutrition of the incubated young. Consumption of nurse eggs by developing young occurs in some coelomic and ovarian brooding sea cucumbers (Turner and Dearborn, 1979; chapter six). Lecithotrophy, where nutritional needs of embryos and young are provided by yolk reserves invested into the eggs by the parent, is the sole mechanism of nutrition discovered so far among externally brooding dendrochirotids (Smith, 1962; Engstrom, 1982; chapter four; Table V-3).

Separating the species in Table V-3 into those exhibiting broodcare and those producing a pelagic, lecithotrophic larva is also a separation of two general body sizes. Assuming body length is indicative of body size and that body length values given in the literature are representative of each species, dendrochirotids with pelagic larvae have body lengths ranging 6.3 - 60 cm ($\bar{x}$ = 18 ± 16 cm, s.d.), while brooding species are significantly smaller ($p<0.01$, Mann-Whitney U-test) at 0.6 - 12 cm length ($\bar{x}$ = 4 ± 3 cm, s.d.). This association of brooding with small adult size is common among marine invertebrates and has also been documented among the Dendrochirotida for the Psolidae (chapter four).

Of those species for which there is information on egg characteristics, only two (Cucumaria lubrica and C. pseudocurata) are known to enclose the egg in a tough vitelline membrane (Smith, 1962; Engstrom, 1982). These two species are also the only forms that practice broodcare outside the maternal body, not utilizing the coelom or epidermal pouches as repositories for developing embryos. With the eggs located outside the female, the tough egg membrane may serve as a protection from bacteria, protozoa, or fungal growth. The membrane could also protect embryos from the pressure exerted on them by the female, especially in the case of C. pseudocurata where the female tenaciously grips the substratum with the tube feet. In intertidal mussel beds exposed to winter rains, a shielding encasement for C. pseudocurata embryos could also reduce mortality from salinity changes. In the event that an egg is broken or an embryo dies, surrounding eggs would be insulated from the effects of decomposition.

Because the continued development of embryos from these two brooders is not dependent on the presence of a brooding parent and, because females of C. lubrica lose a high percentage of eggs during egg deposition (Engstrom, 1982; personal observations), dispersal may well be effected by lost eggs. Here again, the egg membrane could function as a protection for the embryo during its planktonic or benthic development.

PELAGIC LARVAE

A comparison of morphological and behavioral characteristics among dendrochirotid larvae reveals some interesting relationships of potential importance in sea cucumber life-cycles. For those species examined in this study, the general course of development following the gastrula stage is a lengthening of the gastrula into a uniformly ciliated larva. This early larva subsequently shortens in length and at this time can remain evenly ciliated or it can form epidermal ridges (this stage termed the early doliolaria) that will later support the transverse rings of cilia. Patterns of external ciliation assumed by later larvae (whether altered or not from earlier stages) can be used to classify the larvae studied to date (Table V-3): 1) uniform ciliation covering the entire larva, 2) three ciliary rings, and 3) four ciliary rings. Some confusion exists in earlier works with respect to the final number of ciliary rings worn by the larva. Larvae of four of the species I cultured (Pentamera populifera, Cucumaria miniata, C. piperata, and Cucumaria sp.) and of Psolus chitonoides and Psolidium bullatum (chapter four) first form two ciliary rings posterior to the preoral lobe with both the preoral lobe and posterior end retaining uniform ciliation. Cilia are then lost from the posterior end, leaving three transverse rings. Problems have arisen from previous investigators labelling the ciliated preoral lobe and posterior end as rings or bands and also from failure to detect ciliary loss on the posterior end. The latter can be difficult to recognize since it can take place at or just prior to settlement (as in P. chitonoides) for a brief period before cilia disappear after settlement. Although Ohshima (1918) describes "3 and, rarely, 4" ciliary rings for C. echinata, Selenka (1876a,b) occasionally noted five instead of the usual four rings for Ocnus planci, and Jones (1960) says there are 3 - 5 ciliary rings on the larva of P. chitonoides, ciliary ring number was constant for all conspecific larvae in the species I studied.

The varying patterns of ciliation on invertebrate larvae have long intrigued zoologists and have triggered speculation on their significance (reviewed by Chia et al., 1984). Grave (1903) imputed locomotory and feeding functions to the ciliary rings of echinoderm larvae; the latter function, however, is not applicable to holothuroid doliolaria larvae since a feeding doliolaria has not been found. Another potential role for ciliary rings is maintaining larvae suspended that are carrying heavy body parts (Garstang, 1928). This might apply to larvae of sea cucumbers where a delay in settlement has been quite prolonged and ossicle formation has progressed to an advanced stage, but the larvae in this study had barely initiated ossicle growth at the time of settlement. Newly settled young of Cucumaria miniata, C. piperata, and Cucumaria sp. that have lost their cilia and are yet without the heavy coat of calcareous ossicles are probably still positively buoyant, as is the equivalent stage of Psolus chitonoides (chapter four). Larvae of all four of these species develop from floating eggs, further emphasizing the unlikelihood of their being weighted down with calcareous ossicles toward the end of their pelagic period. In these cases, ciliary rings possibly aid the settling larvae to swim against the positive buoyancy centered in the yolky preoral lobe.

A relationship between larval type and adult habitat is indicated from observations of larval settlement sites in the field combined with qualitative assessments of larval behavior in the laboratory. Young and Chia (1982) have already demonstrated that for Psolus chitonoides (whose adults occupy mainly the undersurfaces and sides of rocks), late larvae and early juveniles are strongly photonegative. Other larvae exhibiting negative phototaxis at and following settlement are species whose adults are also encountered underneath boulders and cobble (Cucumaria miniata, C. piperata, and Cucumaria sp.). New settlements of C. miniata and C. piperata were found under rocks among conspecific adult populations at Crane Island. An attraction for light was noticed in laboratory cultures of Pentamera populifera doliolaria larvae which settle in high numbers on top of the mud among adult populations. In comparison, Eupentacta quinquesemita larvae displayed no observable light response during settlement in culture bowls. Recently settled young of E. quinquesemita were encountered on hydroids, algae, and sabellid tubes in adult habitats (rock walls and tops of boulders in high current channels, docks, pilings). The evenly ciliated early larva of P. chitonoides, before ciliary ring formation, was also shown by Young and Chia (1982) to exhibit no phototaxis. Other dendrochirotids with uniformly ciliated larvae (Table V-3) show adult and juvenile distributions not unlike E. quinquesemita: Agassiz and Agassiz (1865), Whiteaves (1901), and Jordan (1972) describe C. frondosa adults covering shallow subtidal rocks and Olaso Toca (1982) noted numerous 2 - 9 mm juveniles of Aslia lefevrei on ropes securing mussel mariculture rafts. Settled young of C. frondosa (another species with a uniformly ciliated larva) were found on floating algae (Jordan, 1972; appendix one). Dendrochirotid larvae with ciliary rings, therefore, appear to have a photoresponse (either positive or negative) that is apparently absent in the uniformly ciliated larva.

From the above, one might predict that for dendrochirotids found on hard substrates, those dwelling under rocks and ledges will produce doliolaria larvae having ciliary rings and exhibiting negative phototaxis in late larval stages. Conversely, those living on tops of rocks or on docks and pilings should have a uniformly ciliated larva lacking phototactic behavior. The association of a light response with ciliary ring formation would imply the development of photoreceptors, which, in these larvae, are probably located in the region of the ciliary rings where the epidermis has been denuded of cilia. Recent autoradiographic studies on the larvae of the crinoid Florometra serratissima have revealed a subepidermal nerve network present in between the ciliary rings of the doliolaria (R. Koss, personal communication).

A complication with the above scheme is that Cucumaria piperata, although abundant under rocks in cobble fields, is somewhat of a generalist with respect to habitat and also occurs in large beds on mud bottoms where it lives in u-shaped burrows or lies on top of the mud (personal observations). More field observations of larval behavior combined with in-depth, quantitative studies documenting ontogenetic changes in behavior like Young and and Chia's (1982) are needed, using closely related species with larvae of differing patterns of ciliation to better understand the relationships among larval morphology and behavior and juvenile and adult distributions.

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VI. REPRODUCTIVE AND DEVELOPMENTAL PATTERNS IN THE HOLOTHUROIDEA (ECHINODERMATA)

A. INTRODUCTION

The single gonad of sea cucumbers is a primitive trait that separates them from other echinoderm classes, but in common with other echinoderms, most holothuroids are dioecious and free-spawning with external fertilization and development. The reproduction of echinoderms has received an increasing amount of attention in the last 40 years with most of the work centering on the echinoids, asteroids, and ophiuroids. Thorson (1936, 1946, 1950) outlined the sexual reproductive patterns for asteroids as pelagic planktotrophic larvae from small eggs, pelagic lecithotrophic larvae from larger eggs, and large eggs with direct development that are usually brooded. The reproductive ecology of ophiuroids was reviewed by Hendler (1975) who placed emphasis on the three major patterns of direct, planktotrophic, and abbreviated development. Hendler's classification scheme was further expanded by Rumrill (1982) to discriminate among larval types and differences in larval ecology. An in-depth review detailing trends in reproduction and development for the class Holothuroidea has not been conducted.

In the past, the reproduction and development of the Holothuroidea have been reviewed in varying degrees of thoroughness by a number of authors (Bronn, 1889-1892, Bury, 1889; Bather et al., 1900; Clark, 1902b; Ludwig, 1904; MacBride, 1914; Ohshima, 1921; Vaney, 1925; Cuenot, 1948; Hyman, 1955; Fell, 1965; Boolootian, 1966; Inaba, 1968). All of these reviews have been hampered by the lack of information, stemming largely from the fact that the majority of studies on sea cucumber reproduction are brief and incomplete. A more thorough, up-to-date summary is also needed since even the most comprehensive treatment by Hyman (1955) has not included much useful information; in addition, the intervening 30 years since her major treatise have yielded more material that requires incorporation with the earlier literature.

This chapter is a comprehensive review of the reproduction and development of holothuroids [illegible] information in the literature with the data contained in the previous four chapters, as well as added details on the reproduction and development of *Parastichopus californicus* and *Leptosynapta clarki*. Each order is taken separately and developmental patterns evident from the available information are outlined and discussed. Also included are sections on breeding seasons, egg size, fecundity, length of pelagic period, and size at metamorphosis. A brief summary of reproductive characteristics of the developmental patterns is then made with a short statement on their possible evolutionary significance.

B. MATERIALS AND METHODS

Sites and methods of collection are the same as described in chapters 2 - 5. Specimens of *Parastichopus californicus* were collected using SCUBA in front of Friday Harbor Laboratories and in East Sound, Orcas Island, at 7 - 20 m depths; *Leptosynapta clarki* were dug up near the mouth of False Bay on San Juan Island.

Maximum egg diameters for *Parastichopus californicus* and *Leptosynapta clarki* were measured for 10 ripe eggs from each of five females; egg sizes for *Cucumaria lubrica*, *C. miniata*, *C. piperata*, *C. pseudocurata*, *Cucumaria* sp., *Eupentacta quinquesemita*, *Molpadia intermedia*, *Pentamera populifera*, *Psolidium bullatum*, and *Psolus chitonoides* were taken from chapters 3 - 5. Maximum egg sizes reported for other holothuroids were used in the literature reviews of the holothuroid taxa. Fecundity estimates for sea cucumbers that free-spawned their eggs in the laboratory (*Cucumaria miniata*, *C. piperata*, *Cucumaria* sp., *Eupentacta quinquesemita*, *Pentamera populifera*, *Psolidium bullatum*, and *Psolus chitonoides*) during their respective breeding seasons (chapter two) were made by collecting all the released eggs of one female with a turkey baster into a 4.4-liter jar of sea water. After thorough mixing, ten 10-ml aliquots of eggs were sampled with a syringe and enumerated. Direct counts of ripe eggs in ovaries were possible for the three brooders *C. lubrica*, *C. pseudocurata*, and *L. clarki*. Due to the high fecundities of female *Molpadia intermedia* and *P. californicus*, and because few of them spawned in the laboratory, ovarian egg number was estimated by measuring the length of the ovarian tubules and then sampling 10 1-cm sections from the ripe tubules for egg counts.

For larval cultures, ripe eggs of *Parastichopus californicus* were obtained by dissecting out ripe ovarian tubules complete with gonoduct, placing them in a large dish with enough filtered seawater to cover the tubules, and waiting until eggs were released into or out of the gonoduct. Other culturing procedures followed those given in chapter three. Auriculariae were fed every 1 - 2 d with Dunaliella tertiolecta and Isochrysis galbana.

Size at metamorphosis was taken as the length from anus to the base of the tentacles on recently settled young juveniles with a newly acquired coat of ossicles. At least eight siblings were measured from two or more females for each species.

C. REPRODUCTION AND DEVELOPMENT

SEASONALITY OF SEXUAL REPRODUCTION

Because of the obvious vital role sexual reproduction plays in the continuance of most life forms, much study has been devoted to elucidation of the ultimate and proximate factors thought to have greatest influence on different phases of reproduction. The majority of marine macroinvertebrates lives in shallow-water habitats that experience periodic fluctuations in such environmental variables as temperature, light, salinity, and food availability. The cyclic nature of gametogenesis and the seasonality of breeding in marine invertebrates are well documented phenomena and have been correlated with a number of these environmental variables (reviewed by Giese and Pearse, 1974). This section treats reproductive periodicity in holothuroids, summarizing the published information on breeding seasons (Table VI-1) and the possible regulating factors.

One of the two most noticeable patterns among the breeding seasons listed in Table VI-1 and summarized in Figure VI-1 is the large number of dendrochirotids (15 species) that breed February through May. These late winter-springtime spawners are concentrated in the cold temperate waters of the northeast Pacific and north Atlantic oceans; they broadcast their gametes and have a pelagic doliolaria. Brooding dendrochirotids either breed continuously or spawn in winter (Fig. VI-2). Gonad index values and histological techniques have been used to follow the reproductive cycles of three species, Sclerodactyla briareus, Cucumaria pseudocurata, and Aslia lefevrei. Turner and Boolootian's (in Boolootian, 1966) gonad indices for S. briareus indicated a winter breeding period, in contradiction to the April to July period shown by nine prior reports based on ripe specimens and laboratory spawnings. The most in-depth study on a dendrochirotid reproductive cycle is by Costelloe (1985)) for A. lefevrei. He showed

Table VI-1. Breeding seasons of the class Holothuroidea arranged according to order (classification following Pawson, 1982). Methods employed by investigators to determine breeding seasons are coded: B = embryos or young being brooded, C = cultures obtained by artificial or natural fertilization (laboratory), E = egg size, F = field spawning(s), G = gonad index, H = histological examination of gonads, L = laboratory spawning(s), P = eggs, embryos, or larvae observed in the plankton, R = ripe gonads, r = ripe gametes (a maturity index sometimes used), Y = release of young, --- = method not indicated. Data in question are numbered (in method column) and explained at end of table.

SPECIES	BREEDING SEASON	METHOD	COLLECTION SITE	SOURCES
DENDROCHIROTIDA				
Aslia lefevrei	Mar	E	England	Orton, 1914
" "	late Jan - early Apr	---	n. w. Spain	J. Costelloe,personal communication
" "	late Feb - early Apr	G,H,L	Galway, Ireland	Costelloe (in press)
Cucumaria echinata	mid June - early Aug	C,L,R	Misaki, Japan	Ohshima, 1918, 1921
C. frondosa	Mar	P	Bergen, Norway	Danielssen & Koren, 1856
" "	July	P	Greenland	Bergendahl (in Runnstrom & Runnstrom, 1918-1919)
" "	Aug	R	Massachusetts	Clark, 1902b
" "	late Feb	P	Bergen, Norway	Nordgaard, 1912
" "	late Feb - late Mar	C,L(?)	North Sea, No. Atlantic	Runnstrom & Runnstrom, 1918-1919
" "	June, July	---	Arctic	Runnstrom & Runnstrom, 1918-1919
" "	late Mar - Apr	C,G,L,P, R	Maine	Jordan, 1972
" "	Jan - Mar	L	Bergen, Norway	Lonning, 1976

" "	late Apr - 3rd wk May	P	New Brunswick	Lacalli, 1981
" "	Mar - May	P	no. Norway	Falk-Petersen, 1982a, b
C. japonica	early Mar - mid Nov (maximum early July - late Sept)	G,R	no. Japan	Kinosita & Sibuya, 1941
C. lubrica	mid Nov - early Jan	B,C,F,L	San Juan Islands	Engstrom, 1982, chapters 2, 5
C. miniata	Mar - May	P',R	San Juan Islands	Johnson, 1931; Johnson & Johnson, 1950
" "	Apr, May	F	" "	M. Strathmann, 1974
" "	early Mar - mid May	C,F,L	" "	chapters 2, 5
C. piperata	Mar - May	R	San Juan Islands	Johnson, 1931; Johnson & Johnson, 1950
" "	late Feb - mid May	C,F,L	" "	chapters 2, 5
C. pseudocurata	Dec	B	central California	MacGinitie & MacGinitie, 1949
" "	Jan - Apr	B'	" "	Smith, 1962
" "	late Dec - early Jan	B	" "	Brumbaugh, 1964
" "	Jan	B,G,r	" "	Rutherford, 1973
" "	"	B	" "	Turner & Rutherford, 1976b
" "	mid Dec - early Jan	B,C,L	San Juan Islands	chapters 2, 5
C. tergestina	Dec - Jan	---	Trieste	Graeffe, 1881(from Bronn, 1889-1892
Cucumaria sp.	late Mar - late May	C,F,L	San Juan Islands	chapters 2, 5
Eupentacta quinquesemita	Mar - May	P',R	San Juan Islands	Johnson, 1931; Johnson & Johnson, 1950
" "	Feb - May	G,L	Vancouver Island	Byrne, 1983

" "	early Mar - early June	C,L	San Juan Islands	chapters 2, 5
Leptopentacta elongata	June - July	---	England	Mar. Biol. Assoc., 1931
" "	Jan	C,L	"	Chia & Buchanan, 1969
L. panamica	Jan	R	w. Mexico	Deichmann, 1941
Lissothuria antillensis	Feb, May, July, Oct (probably continuous)	B	Bermuda, Caribbean	Miller 1983, 1985
L. nutriens	summer	B	central California	Clark, 1901, 1902b
" "	Mar - Nov (probably continuous)	B	" "	Deichmann, 1941
" "	continuous	B,L,r	" "	Wootton, 1949
" "	June, Aug	B	" "	MacGinitie & MacGinitie, 1949
Neoamphicyclus lividus	Dec	B	Tasmania	Hickman, 1978
Neocnus incubans	Apr	B	Tunisian coast	Cherbonnier, 1972
Ocnus planci	Mar, Apr	C	Naples	Selenka, 1876 (from Hyman, 1955
" "	Sept - May, Mar - Apr	---*	Naples, Trieste	Lo Bianco, Ersig, Selenka, Schmidtlein, Graeffe (in Bronn, 1889-1892)
" "	late June	L	Puffin Island, Wales	Chadwick, 1914
" "	Mar - Apr	R	Bahamas	Deichmann, 1957
Pachythyone lugubris	July	B	Lower California	Deichmann, 1939
P. rubra	July	B	central California	MacGinitie & MacGinitie, 1949
" "	continuous	V	" "	Chaffee, 1982
Pawsonia saxicola	May	E	England	Orton, 1914
" "	Mar - May	L,R	"	Mar. Biol. Assoc., 1931

Pentamera chiloensis	late Jan	R	Galapagos	Deichmann, 1941
P. populifera	mid Feb - mid May	C,F,L	San Juan Islands	chapters 2, 5
P. pulcherrima	late winter, early spring	R	Woods Hole	Clark, 1902b
Phyllophorus conchilegum	May	R	Florida	Deichmann, 1930
P. urna	June, summer	---	Naples	Kowalevsky, Schmidtlein (in Bronn, 1889-1892)
" "	June	B	"	Ludwig, 1898
Pseudopsolus macquariensis	May - June	B,E,R,r	Macquarie Island	Simpson, 1982
Psolidium bullatum	early Mar - late May	C,F,L	San Juan Islands	chapters 2, 4
Psolus chitonoides	Mar - May	R,P	San Juan Islands	Johnson, 1931; Johnson Johnson, 1950
" "	mid Mar - early June	C,L,P	" "	Jones, 1960
" "	Apr	C,L	" "	Young & Chia, 1982
" "	early Mar - early June	C,F,L	" "	chapters 2, 4
P. ephippifer	Feb	B	Antarctic	Thomson 1878a, b
P. phantapus	Mar, Apr	C,P	North Sea	Runnstrom & Runnstrom, 1918-1919
" "	Mar - mid summer	P(?)	British Isles	Mortensen, 1927
" "	Feb - Mar	L,P	Bergen, Norway	Thorson, 1946
Sclerodactyla briareus	June, July	---	Woods Hole	Bumpus, 1898
" "	Apr	R	" "	Mead, 1898
" "	summer	---	" "	Clark, 1902b
" "	late June - early July	L	" "	Pearse, 1909
" "	June	r	Connecticut	Coe, 1912

" "	June	L,C	Woods Hole	Ohshima, 1925
" "	"	L	" "	Just, 1929
" "	"	L,R	" "	Colwin, 1948
" "	June	L,r	" "	Colwin & Colwin, 1956
" "	winter	G	Cape Cod, Massachusetts	Turner & Boolootian (in Boolootian, 1966); Turner, 1966
Stereoderma kirschbergi	summer	C	Mediterranean	Kowalevsky, 1867
Thyone fusus	Mar - Apr	---	Trieste	Graeffe, (in Bronn, 1889-1892)
" "	May - June	---	Scotland	Dalyell (in Bronn, 1889-1892)
" "	Mar - May	G,L	Galway, Ireland	J. Costelloe, personal communication
T. inermis	May - June	---	Trieste, Naples	Lo Bianco, Graeffe (in Bronn, 1889-1892)
DACTYLOCHIROTIDA				
Ypsilothuria talismani	continuous	E,H	Rockall Trough (No. Atlantic)	Tyler & Gage, 1983
ASPIDOCHIROTIDA				
Actinopyga echinites	Jan, Feb	G,R	New Caledonia	Conand, 1982
A. mauritiana	May	L,C	Red Sea	Mortensen, 1937
A. serratidens	May	C,L	Red Sea	Mortensen, 1937
Bathyplotes natans	winter (some individuals all year?)	E,R	Norway	Lonning, 1976
Bohadschia marmorata	May	C,L	Red Sea	Mortensen, 1937
" "	Dec	F	New Guinea	Shelley, 1981

" "	July - Aug	F	Palau	Hendler & Meyer, 1982
Holothuria arenicola	May	C,L	Red Sea	Mortensen, 1937
H. atra	last half of July	R	Misaki, Japan	Colwin & Colwin, 1955
" "	continuous	E,H,R,r	Red Sea	Pearse, 1968a
" "	May-June, Dec-Jan (semi-annual)	G,H	Great Barrier Reef	Harriott, 1980, 1982
H. difficilis	Aug	C,L	Red Sea	Mortensen, 1938
H. edulis	continuous	---	Great Barrier Reef	Harriott, 1980, 1982
H. floridana	mid July - mid Aug	C,R	Bahamas	Edwards, 1889, 1907, 1908-1909, 1909
" "	mid Sept - late Oct	G,H,L	Florida	Engstrom, 1980a
H. forskali	Apr, July, Aug, Dec	P,R	England	Mar. Biol. Assoc., 1931
H. glaberrima	June, July	C	Barbados	Lewis, 1960
" "	Aug	E,R	Bahamas	F. S. McEuen, unpublished data
H. impatiens	July	C,L	Red Sea	Mortensen, 1938
" "	spring	---	Great Barrier Reef	Harriott, 1980, 1982
H. kefersteini	Oct - Nov	C,r	Bay of Panama	Mortensen, 1921
H. leucospilota	summer	R	Red Sea	Pearse, 1968a, b
" "	June, July	F	Okinawa	Toma, 1980
" "	warm season	---	Great Barrier Reef	Franklin, 1980 (in Conand, 1982)
" "	July - Aug	C,r	Seto Mar. Lab., Japan	Maruyama, 1980
H. mexicana	mid Aug - late Oct	G,H,L	Florida	Engstrom, 1980a
" "	mid July - mid Sept	F,L	Bahamas	Mosher, 1982

H. moebi	late July - Aug	r	Japan	Noumura & Kanatani, 1962 (in Boolootian, 1966)
H. papillifera	Aug	C,L	Red Sea	Mortensen, 1938
H. pardalis	late Sept	C	Red Sea	Mortensen, 1938
" "	July - Aug	C,r	Seto Mar. Lab., Japan	Maruyama, 1980
H. poli	Nov - Jan	---	Naples, Trieste	Lo Bianco, Graeffe (in Bronn, 1889-1892)
" "	late July - Aug	C	Naples	Horstadius, 1925, 1939
H. scabra	June	C,L	Red Sea	Mortensen, 1937
" "	continuous, with peaks in July & Oct	G,R	so. India	Krishnaswamy & Krishnan, 1967
" "	Nov - Dec (some throughout year)	F,G,r	New Guinea	Shelley, 1981
H. spinifera	June	C,L	Red Sea	Mortensen, 1937
H. tubulosa	continuous, with maximum in Aug, Sept	C,R	Naples	Selenka, 1876a,b
" "	June - Aug, Feb - Oct, July - Aug	---	Naples, Trieste, St. Tropez	Lo Bianco, Graeffe, Selenka (in Bronn, 1889-1892)
" "	Aug	R,r	Spain	Pladellorens & Subirana 1975
Holothuria sp. (?)	Apr	F	Sormiou	Cousteau & Dugan, 1963
Holothuria n. sp. (?)	Oct	C,r	Gulf of Panama	Mortensen, 1921
Mesothuria intestinalis	Aug	r	Norway	Nordgaard, 1912
" "	continuous (?)	R	Norway	Lonning, 1976
Microthele fuscogilva	Jan - Feb	G,R	New Caledonia	Conand, 1981
M. nobilis	July, Aug	C,L	Red Sea	Mortensen, 1938
" "	Apr	F	Aldabra	Sloan et al., 1979

" "	May - July	G,R	New Caledonia	Conand, 1981
Paelopatides sp.	continuous, with maximum in spring	E,G,H	Oregon	Hufford, 1968
Parastichopus californicus	mid July (fertilization)	C	Puget Sound	Bovard & Osterud, 1918
" "	June	C,r	Vancouver Island	Mortensen, 1921
" "	late June - mid Aug	F,R	San Juan Islands	Courtney, 1927
" "	spring months, May	C	" "	Johnson, 1931; Johnson & Johnson, 1950
" "	Aug	---	Newport Bay, California	MacGinitie & MacGinitie, 1949
" "	June	L(?),r	San Juan Islands	Strathmann & Sato, 1969
" "	early summer	C,r	" "	M. Strathmann, 1974
" "	late May - mid July	L,R,r	" "	Smiley, 1984
" "	early Apr - early Aug	C,F,L,R	" "	chapter 2
Stichopus chloronotus	bi-annual	---	Papua New Guinea	Franklin, 1980 (in Conand, 1982)
S. japonicus	mid May - late July	R,r	Kanagawa, Japan	Mitsukuri, 1903
" "	July - Aug	r	Japan	Kinosita & Sibuya, 1936a,b, 1938 (in Tanaka, 1958)
" "	mid June - mid Aug	G,H,r	so. Hokkaido, Japan	Tanaka, 1958
" "	Apr - Aug	---	Japan	Choe, 1963 (in Boolootian, 1966)
" "	Mar - Sept	---	Japan	Suguri, 1965
" "	early Apr - early Aug	---	Aichi, Mie (Japan)	Funada, 1970
" "	May - June	---	Mutsu Bay, Japan	Funada, 1970

" "	early May - mid May	---	Shimane, Japan	Oshima & Takeuchi, 1977
S. regalis	late Apr	---	Naples	Schmidtlein, 1878 (in Bronn, 1889-1892)
S. tremulus	mid July	L	Norway	Nordgaard, 1912
" "	Aug - Sept	---	---	Runnstrom 1924 (from Lonning, 1976)
" "	late July - Aug	C,L	Norway	Rustad, 1938
" "	summer	R,r	Bergen, Norway	Lonning, 1976
" "	July	C,L	Sweden	Holland, 1981
S. variegatus	June	C,L	Red Sea	Mortensen, 1937
Thelenota ananas	Dec - Apr	G,R	New Caledonia	Conand, 1981
ELASIPODIDA				
Benthogone rosea	continuous	E,H	Rockall Trough (No. Atlantic)	Tyler et al., 1985a,b
Laetmogone violacea	continuous	E,H	Rockall Trough (No. Atlantic)	Tyler et al., 1985a,b
Oneirophanta mutabilis affinis	May (Dec - May?)	B,E,H	Pacific coast, Central America	Hansen, 1968, 1975
APODIDA				
Chiridota rotifera	early Sept	---	Rio de Janeiro	van Beneden (in Bronn, 1889-1892)
" "	Apr, July, Aug	B	Bermuda, Jamaica	Clark, 1910
Epitomapta roseola	Apr	r	Woods Hole	Mead, 1898
" "	late Apr - Aug	R	" "	Clark, 1899
" "	summer	C,R	Massachusetts, Long Island, Bermuda	Coe, 1912

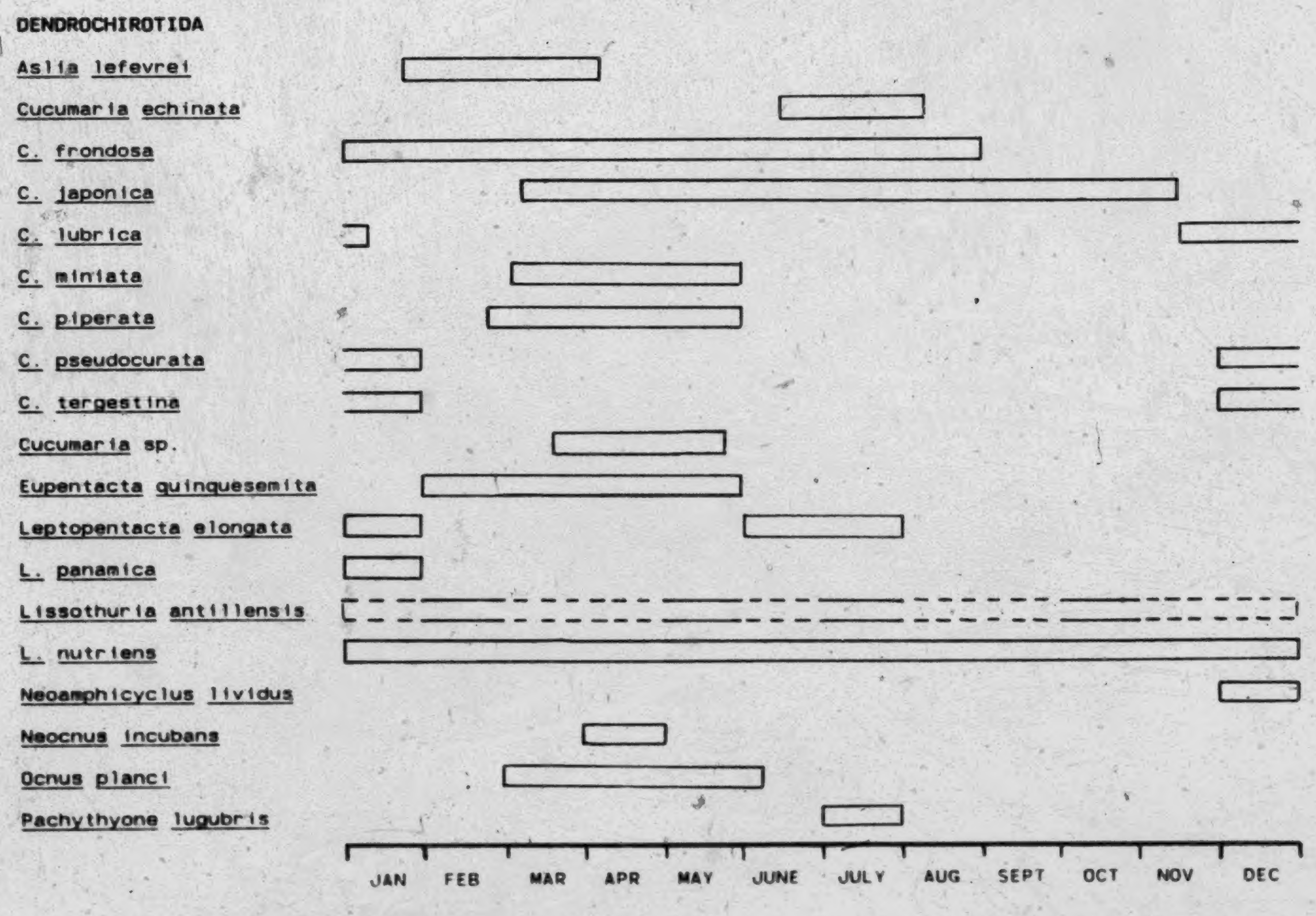
Labidoplax buskii	Oct - Dec	C,L,R,r	Sweden	Nyholm, 1951
Labidoplax digitata	Feb - Apr	---	Naples, Trieste	Lo Bianco, Leydig, Baur, Graeffe, Selenka, Semon (in Bronn, 1889-1892)
" "	Mar, Apr	P	Mediterranean	Reimers, 1912
" "	July	P	Port Erin Bay (Isle of Man)	Chadwick, 1914
" "	summer	---	Plymouth to Shetland Is.	Mortensen, 1927
Leptosynapta clarki	"phases of reproduction passed early in the summer"	r'	San Juan Island	Bovard & Osterud, 1918
" "	late Oct - mid Dec	B,L	San Juan Island	Everingham, 1961; chapter 2
" "	Feb - Apr	B,E,R	Vancouver Island	Brooks, 1973
L. gallienei	May - Sept	L	France	Cherbonnier, 1953
L. girardii	Apr	r	Woods Hole	Mead, 1898
L. inhaerens	June	C,L	Belfast	Thomson, 1862
" "	Oct, Mar	---	Naples	Lo Bianco, 1888 (in Runnstrom, 1927)
" "	Aug - Sept	C,R	Norway	Runnstrom, 1924, 1927
" "	Mar, Apr	L,R	England	Mar. Biol. Assoc., 1931
" "	Aug, Sept	---	Norway	Hyman, 1955
L. minuta	Jan	B	Duslen (France)	Cherbonnier, 1953
L. tenuis	June, July	---	Woods Hole	Bumpus, 1898
" "	late Apr - Aug	R	" "	Clark, 1899
" "	late spring, early summer	R	Long Island Sd.	Coe, 1912

" "	spring, early summer, fall	P	No. Carolina	McCrary, 1969
" "	May - June, Sept - Nov	E,H,R	" "	Green, 1976, 1977, 1978
L. transgressor	Mar - Apr	F	Vancouver Island	Brooks, 1973
Opheodesoma grisea	Aug	C,r	Red Sea	Mortensen, 1938
O. spectabilis	mid June - late Sept	F,R	Hawaii	Berrill, 1966
Rhabdomolgus ruber	Apr - May	E,H,R	Helgoland	Menker, 1970
Synaptula hydriformis	July - Aug	---	Abrolhos, Brazil	Chierchia (in Bronn, 1889-1892)
" "	late Apr - mid July	B	Jamaica	Clark, 1896a,b, 1898
S. reciprocans	May	C,L	Red Sea	Mortensen, 1937
S. vittata	June, July	C,L	Red Sea	Mortensen, 1937, 1938
Trochodota dunedinensis	Sept	B	New Zealand	John, 1939
MOLPADIIDA				
Caudina arenata	Feb - Apr	H,L,R	Massachusetts	Gerould, 1896
Eupyrgus scaber	Aug	r	Arctic	Heding, 1935
Molpadia intermedia	early Nov - early Jan	C,L,R	San Juan Islands	chapter 2
M. roretzii	early winter (Nov or Dec)	R	Onagawa, Japan	Hatanaka, 1939
Paracaudina chilensis	mid May - late June	C,L,P	Asamushi, Japan	Inaba, 1930
" "	Apr - May	---	Mutsu Bay, Japan	Tao, 1930

---

1 - misidentification of larval type (see chapters four and five).
2 - in contradiction with thorough study by Rutherford (1973) and other four studies of this species.
3 - this breeding season supposed by Bovard and Osterud (1918) since they failed to find ripe eggs in July.
4 - the Sept - May season is doubted since it conflicts with the other four reported seasons.

Fig. VI-1. Breeding seasons of the Holothuroidea, based on Table VI-1. Shaded regions of *Holothuria atra* and *Leptosynapta tenuis* are semi-annual breeding seasons. Dotted lines are extended breeding seasons suggested by the original author(s).


DENDROCHIROTIDA
Aslia lefevrei
Cucumaria echinata
C. frondosa
C. japonica
C. lubrica
C. miniata
C. piperata
C. pseudocurata
C. tergestina
Cucumaria sp.
Eupentacta quinquesemita
Leptopentacta elongata
L. panamica
Lissothuria antillensis
L. nutriens
Neoamphicyclus lividus
Neocnus incubans
Ocnus planci
Pachythyone lugubris
JAN
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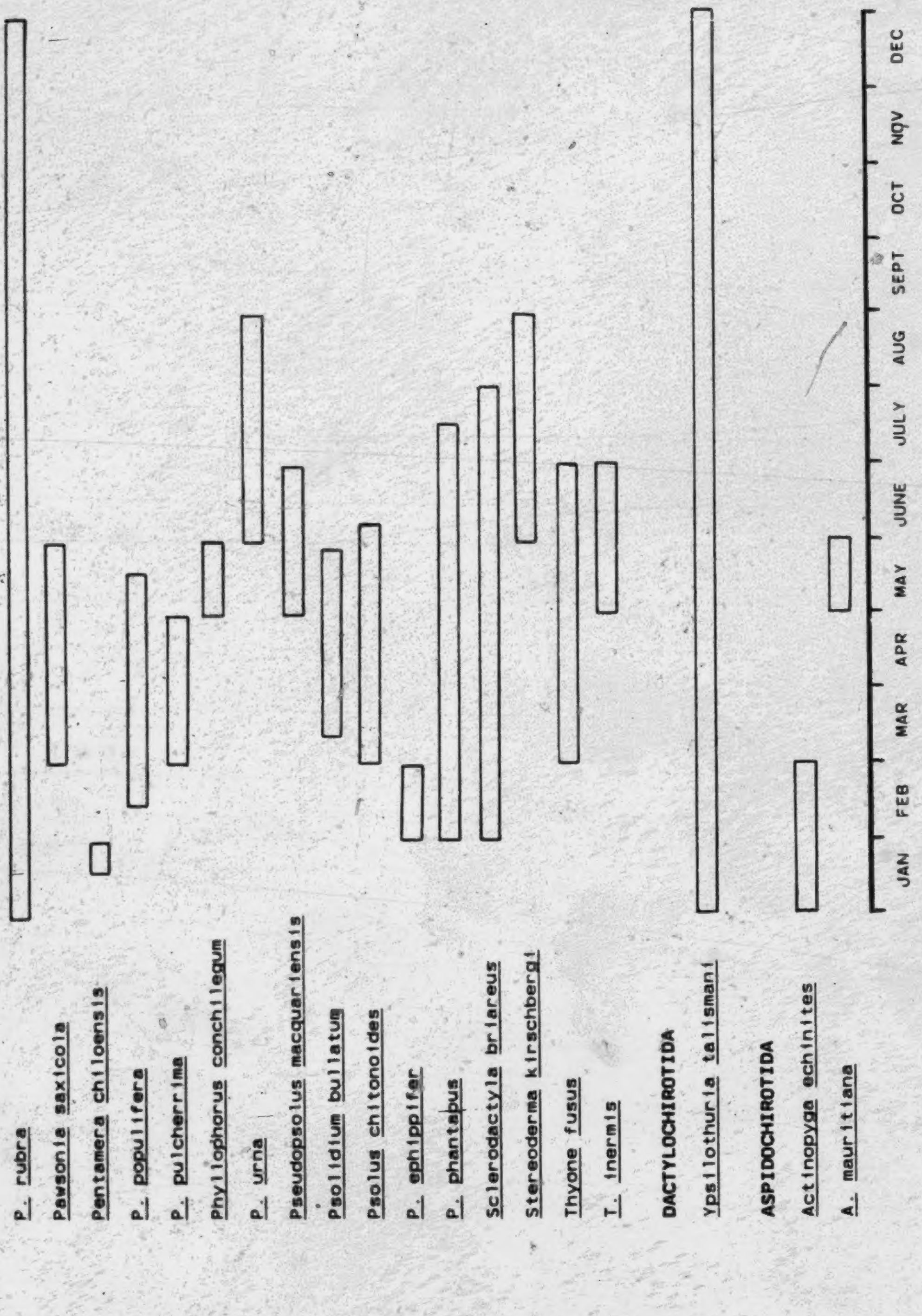
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P. urna
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Psolidium bullatum
Psolus chitonoides
P. ephippifer
P. phantapus
Sclerodactyla briareus
Stereoderma kirschbergi
Thyone fusus
T. inermis
DACTYLOCHIROTIDA
Ypsilothuria talismani
ASPIDOCHIROTIDA
Actinopyga echinites
A. mauritiana
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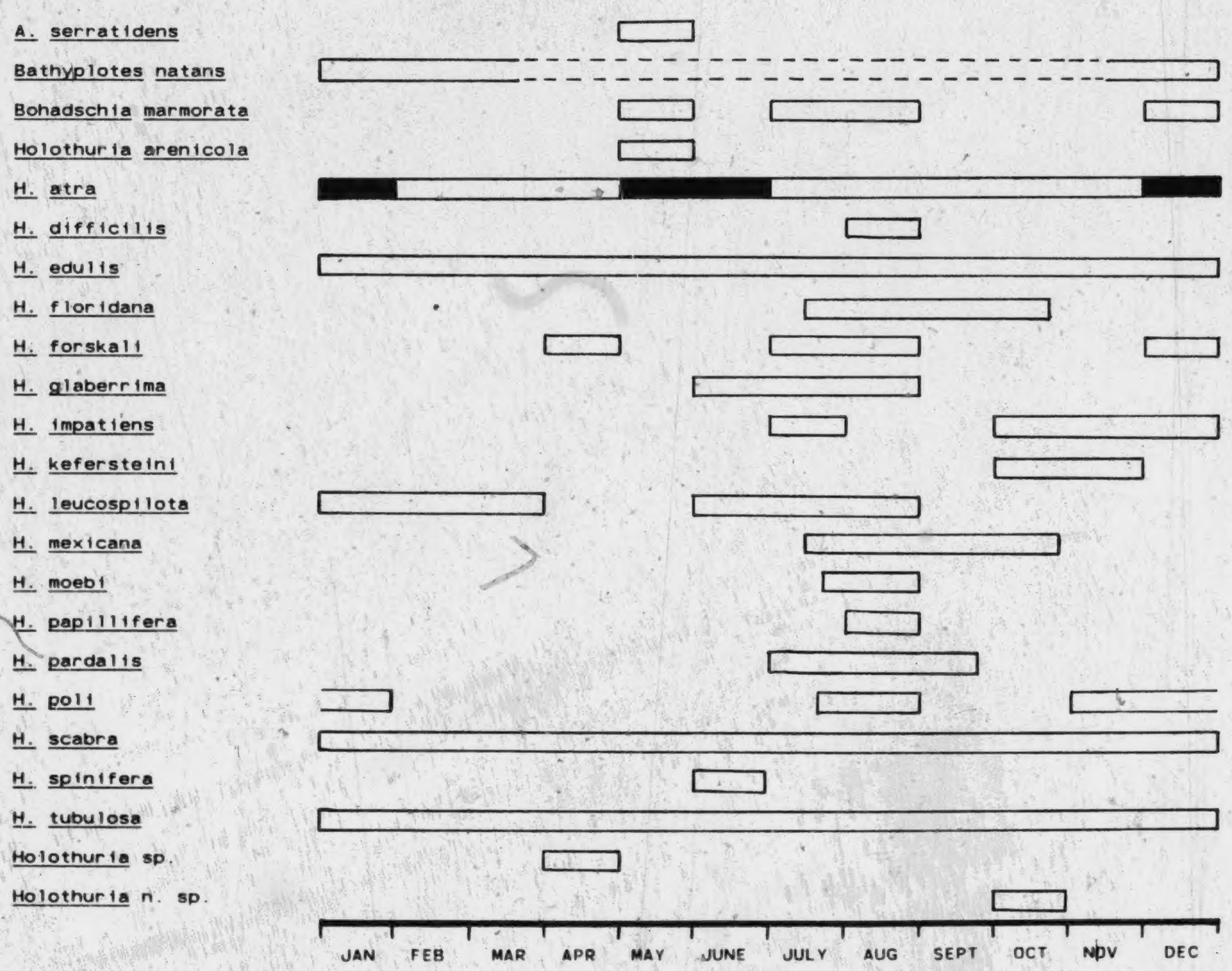
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Bathyplotes natans
Bohadschia marmorata
Holothuria arenicola
H. atra
H. difficilis
H. edulis
H. floridana
H. forskali
H. glaberrima
H. impatiens
H. kefersteini
H. leucospilota
H. mexicana
H. moebi
H. papillifera
H. pardalis
H. poli
H. scabra
H. spinifera
H. tubulosa
Holothuria sp.
Holothuria n. sp.
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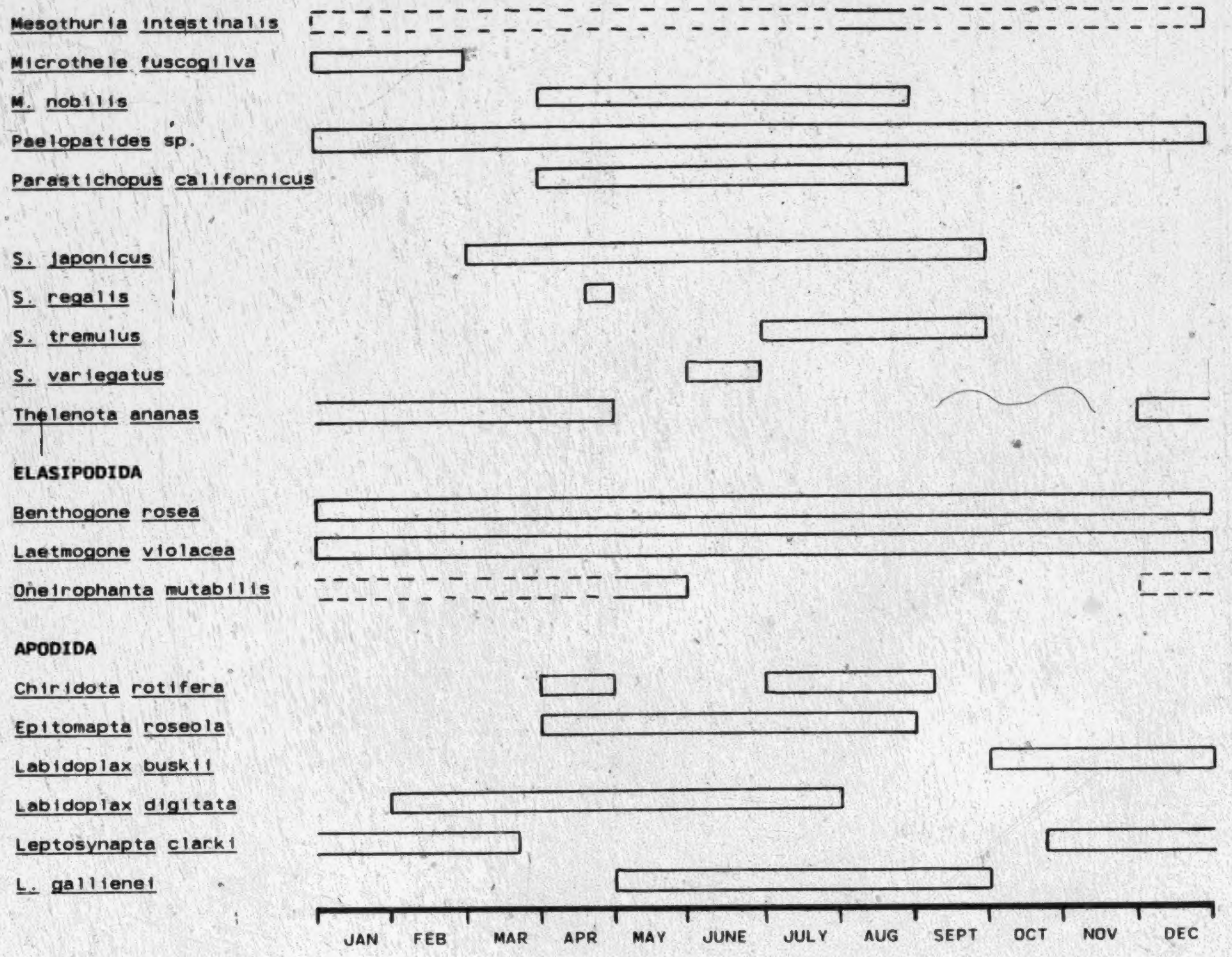
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M. nobilis
Paelopatides sp.
Parastichopus californicus
S. japonicus
S. regalis
S. tremulus
S. variegatus
Thelenota ananas
ELASIPODIDA
Benthogone rosea
Laetmogone violacea
Oneirophanta mutabilis
APODIDA
Chiridota rotifera
Epitomapta roseola
Labidoplax buskii
Labidoplax digitata
Leptosynapta clarki
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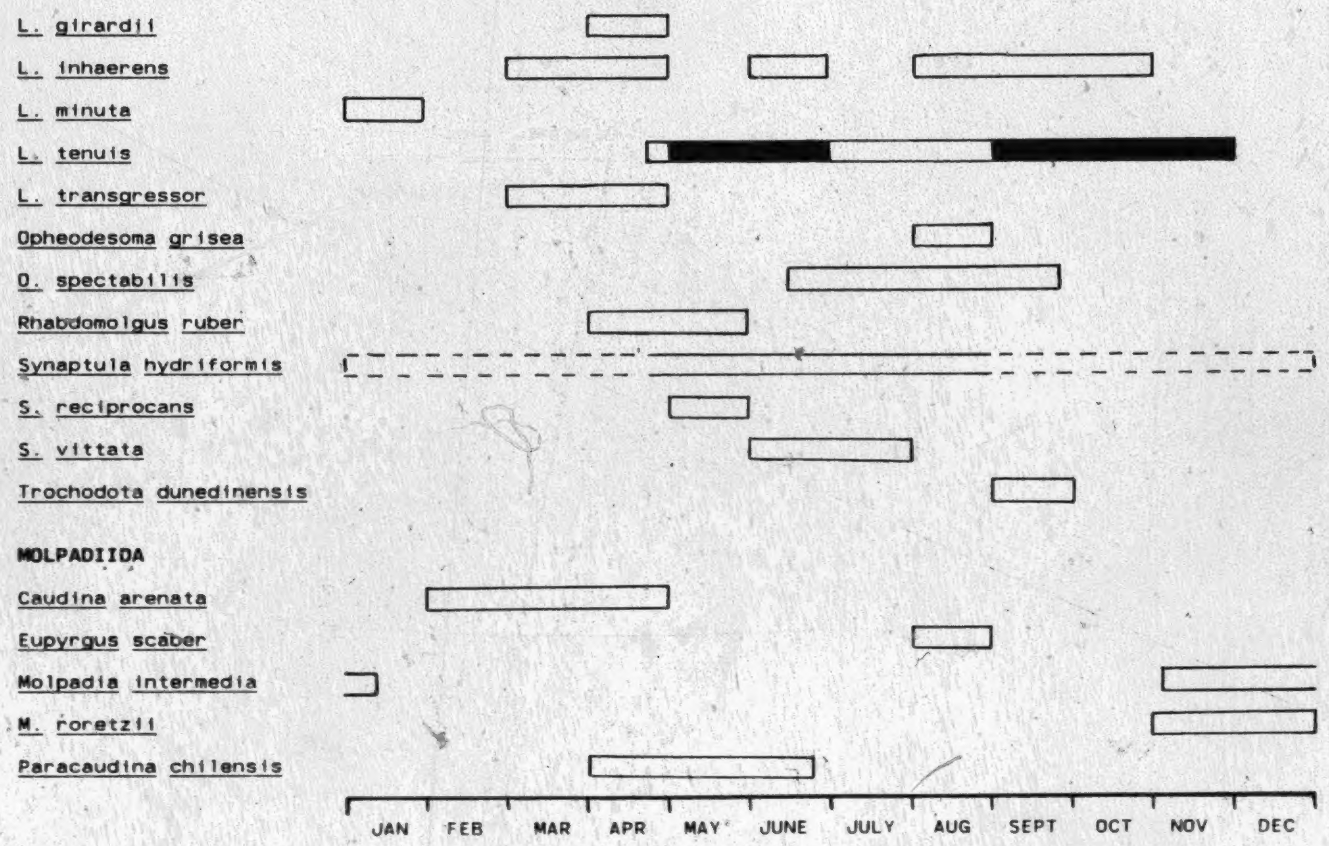
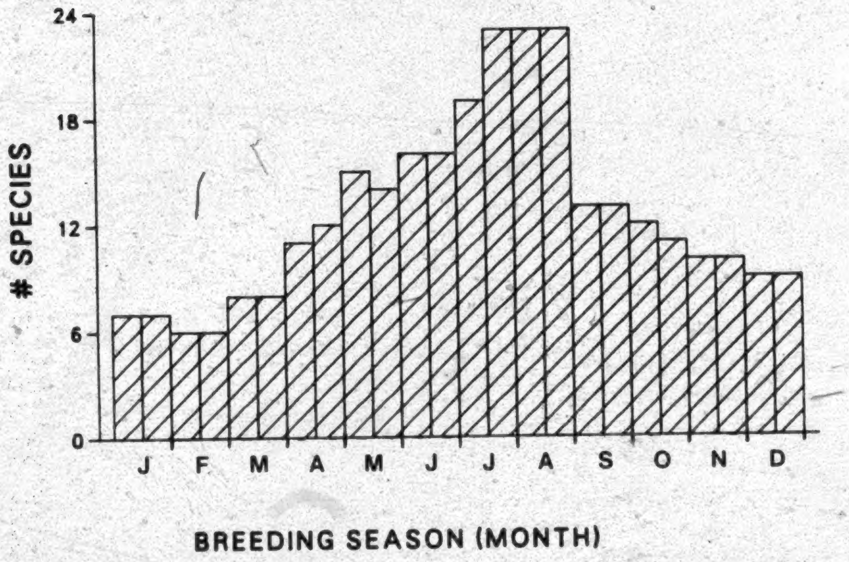
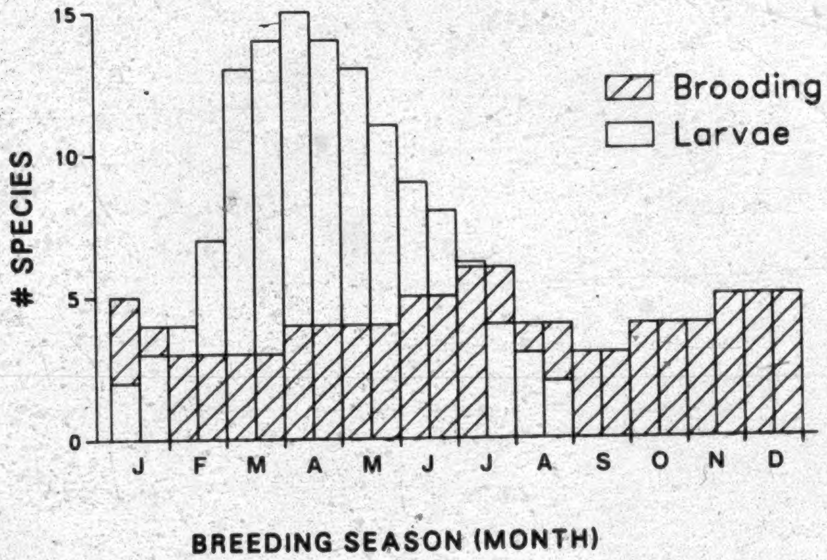

L. girardii
L. inhaerens
L. minuta
L. tenuis
L. transgressor
Opheodesoma grisea
O. spectabilis
Rhabdomolgus ruber
Synaptula hydriformis
S. reciprocans
S. vittata
Trochodota dunedinensis
MOLPADIIDA
Caudina arenata
Eupyrgus scaber
Molpadia intermedia
M. roretzii
Paracaudina chilensis
JAN
FEB
MAR
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC

Fig. VI-2. Histograms of holothuroid breeding seasons by month. The greatest number of species of the Aspidochirotida (top) spawn in summer. Dendrochirotid species (bottom) producing pelagic lecithotrophic larvae (and pelagic eggs with direct development) breed primarily in spring months, while most brooders are found with young year-round. Breeding seasons are according to warm and cold seasons in the northern hemisphere. Breeding seasons for southern hemisphere species were displaced six months to coincide with the warm and cold seasons of the north hemisphere. The months of austral summer (January, February, March), fall (April, May, June), winter (July, August, September), and spring (October, November, December) were based on seawater temperature data of Conand (1982).



Aspidochirotida



Dendrochirotida

nutrient buildup took place over the warmer months and also correlated low winter water temperatures and high dissolved oxygen concentrations with gametogenic activity. Because so many dendrochirotids spawn during spring phytoplankton blooms (Thorson, 1946; chapter two), increased food for newly settled young may be an ultimate cause for their reproductive seasonality ("Crisp's Rule"; Qasim, 1956).

Food for juveniles could also be one factor selecting for winter brooding in high latitudes. There are reliable data for the time when brooding commences in three species. Two species in the northeast Pacific (*Cucumaria lubrica* and *C. pseudocurata*) and one in the subantarctic (*Pseudopsolus macquariensis*) lay their eggs in winter; the first hatchlings of the first two species begin to come out from underneath the females 2 - 4 wks before the spring increase in phytoplankton production (Engstrom, 1982; chapters two and five). Another potential force selecting for brooding in the two *Cucumaria* species to begin in late November to early January, is a tidal series giving long periods of slack water that are favorable for sperm dispersal and fertilization (Engstrom, 1982; chapter two). An intraspecific latitudinal gradient in breeding seasons is evident for three species (*Aslia lefevrei*, *Psolus phantapus*, *Thyone fusus*, and possibly *Ocnus planci*), with more northern populations spawning later (late spring, summer) and southern populations breeding earlier (late winter, spring).

Dendrochirotids in the Mediterranean (*Phyllophorus urna*, *Stereoderma kirschbergi*, and *Thyone inermis*) and Japan (*Cucumaria echinata* and *C. japonica*) reproduce in summer months, but no environmental factors regulating their reproduction have been proposed. Care must be taken with the indicated breeding seasons for some of the dendrochirotids listed in Table VI-1 since a "ripe" condition was sometimes based solely on a one-time qualitative estimate of oocyte size or fullness of the gonad. Because of the large diameters of ripe dendrochirotid eggs, developing oocytes in the unripe gonad may appear quite large in comparison to the small eggs produced by other echinoderms. Also, size of the gonad increases substantially towards the end of the feeding period, 4 - 6 months before the breeding season (Rutherford, 1973; Costelloe, in press; personal observations).

Time of breeding among the shallow-water Aspidochirotida is predominantly during the warm season; at least 23 species spawn in the early to late summer months (Fig.

VI-2). A single species, *Microthele nobilis*, has a cold season spawning period. Of the three stichopodids that live in northern latitudes (*Parastichopus californicus*, *Stichopus japonicus*, and *S. tremulus*), *S. japonicus* has been shown to release gametes as early as May in Kanagawa (central Japan) and into late July in Hokkaido (northern Japan).

Contrasting with the annual reproductive modality are bi-annual and continuous cycles. Species exhibiting two spawning peaks within a year are *Holothuria atra*, *H. scabra*, and *Stichopus chloronotus*; continuous breeding is known for *H. atra* (Red Sea) and *H. edulis*. In the aforementioned species with bi-annual and continuous reproduction, Conand (1982) has noted that binary fission plays an important part in their life cycles.

The most reliable information on aspidochirotid reproductive cycles comes from Tanaka (1958), Krishnaswamy and Krishnan (1967), Pearse (1968a), Engstrom (1980a), Franklin (1980), Harriott (1980, 1982), and Conand (1981, 1982), who employed gonad index values and stages of gonad maturity for assessment of reproductive condition. Temperature (Tanaka, 1958; Conand, 1981, 1982) and salinity changes brought on by monsoon rains (Krishnaswamy and Krishnan, 1967) have been related to the onset of breeding.

No strong trends are readily apparent among the apodids, mostly due to conflicting reports and want of detailed studies. The large tropical and subtropical synaptids (*Opheodesoma grisea*, *O. spectabilis*, *Synaptula reciprocans*, and *S. vittata*), though, do appear to breed in the summer while there are 5 - 6 more northern forms that reproduce in spring or summer. A rise in fjord bottom water temperature coincides with spawning in the meiofaunal species *Labidoplax buskii* (Nyholm, 1951) and high summer temperatures may possibly inhibit gametogenesis in *Leptosynapta tenuis* (Green, 1976, 1978).

In the deep sea, Tyler et al. (1982) have correlated aseasonal reproduction in echinoderms with large egg size, and small eggs with seasonality in breeding. There are 10 deep-sea holothuroids for which there is information on the sexual cycle. *Stichopus tremulus*, *Molpadia intermedia*, and *Paracaudina chilensis* have their main distributions at bathyal or abyssal depths, but the data collected for their breeding seasons cannot be considered here as representative of the deep-sea environment since they were gathered from shallow-water populations. A dactylochirotid (*Ypsilothuria talismani*) and two elasipodids (*Benthogone rosea* and *Laetmogone violacea*) are continuous breeders, there are indications that two synallactids, *Bathyplotes natans* and *Mesothuria intestinalis*, are also aseasonal. *Paelopatides* sp. has a continuous cycle with a spring maximum. Hansen (1968, 1975), using the evidence of either large young or no young at all in the ovaries of *Oneirophanta mutabilis affinis*, suggested that brooding was periodic in this elasipodid and correlated with the rich plankton production during the December to May upwelling season off Costa Rica. The last two cases, though not completely conclusive, emphasize the likelihood that deep-sea echinoderms lacking planktotrophic larvae can be seasonal in their reproduction.

In addition to the fissiparous aspidochirotids, there is another distinct group of continuously breeding sea cucumbers. Three dendrochirotids (*Lissothuria antillensis*, *L. nutriens*, and *Pachythyone rubra*) and an apodid (*Chiridota rotifera*) are small coelomic brooders distributed in warm temperate to tropical regions in littoral and very shallow habitats. *Synaptula hydriformis*, a coelomic-brooding apodid living on mangrove roots, may also belong to this category. The most obvious explanation for constant production of low numbers of young would seem to be the constraints small body size places on gamete output and the harsh environment in which these holothuroids live.

In short, the large majority of sea cucumbers display annual reproductive periodicity, with cold-water dendrochirotids breeding in spring (and some in summer), and summer reproduction most common for warm-water aspidochirotids. Bi-modal and aseasonal reproduction are associated with fissiparity in aspidochirotids and with coelomic brooding in dendrochirotids and apodids; these holothuroids are mostly inhabitants of the stressful intertidal zone and reef flat pools in low latitudes. Non-cyclic breeding activity may also prevail in the deep-sea forms, presumably as a result of the uniform environmental conditions, but no definite statement can yet be made considering so few species have been studied.

The total number of detailed studies on the seasonality of holothuroid reproduction is still very small. The complexity of the phenomena associated with following sea cucumber cycles has been emphasized by Conand (1981, 1982) who stressed the combined use of variation in gonad index, the cycle of different maturity stages, and the percentage of undetermined individuals to obtain a coherent outline of the sexual cycle. According to those who have examined holothuroid breeding cycles, the most likely environmental variables involved with synchronizing reproductive events in holothuroids are temperature, salinity, oxygen concentration, and current (tidal) regimes, but there has been no experimentation to identify them.

PATTERNS OF DEVELOPMENT

Dendrochirotida

The first recognized classical descriptions of the doliolaria larva of the Dendrochirotida are the works of Danielssen and Koren (1856), Kowalevsky (1867), and Selenka (1876a,b). Up to the turn of the century, however, attention was mainly directed to study of the auricularia (see below), and it was not until the early part of this century (Ohshima, 1918, 1921) that a complete sequence of developmental stages from fertilization through metamorphosis was known for a dendrochirotid with a pelagic larva. A classification of the different larval types in this order using the nineteenth century literature does not leave a clear picture, owing to the brevity of the investigations, errors in description, and depiction of odd-shaped larval stages that have not been observed by subsequent workers. A number of short notes on brooding species collected during expeditions were also published towards the close of the last century (see Table V-3). This consideration of dendrochirotid development patterns, then, is based for the most part on the more recent studies.

Reviews of development for the family Psolidae and the six other dendrochirotid families (Placothuriidae, Paracucumidae, Heterothyonidae, Phyllophoridae, Sclerodactylidae, and Cucumariidae) are found in chapters four and five, respectively. Combining the information in those two chapters, developmental types in this order can be separated into two major divisions: pelagic and brooded. Further subdivisions under these two headings are as follows:

Pelagic development:

1) doliolaria with four ciliary rings (two spp.; Ocnus planci, reported to brood eggs briefly among the tentacles, is included here);
2) doliolaria with three ciliary rings (nine spp.);
3) uniformly ciliated larva (five spp.), and;

4) pelagic egg with direct development producing a pentactula at hatching (two spp.).

Brooded development:

1) underneath sole (or trivium) against rock substratum (five spp.);
2) in pockets, folds, depressions, or swellings of sole (three spp.);
3) dorsal depressions (one sp.);
4) brood chambers beneath dorsal calcareous plates (one sp.);
5) interradial pouches surrounding crown of tentacles:
    a) two ventral pouches (six spp.),
    b) 1 - 3 dorsal pouches (six spp.),
    c) five pouches, one per interambulacrum (two spp.), and;
6) in the coelom (six spp.; one of these species with uniformly ciliated larvae in the coelom).

Not included above are three additional brooding species for which details are lacking on the manner of incubation, and one unspecified free-swimming larval type.

Development, then, for these 52 dendrochirotids is composed of 33 brooders (63%) and 19 species with free-swimming larvae or pelagic eggs (37%). Large, yolky eggs are a major feature in this order and maximum egg sizes for the species above with known development are 200 - 1800 um.

Dactylochirotida

This is a recently proposed order (Pawson and Fell, 1965) composed of certain genera formerly grouped with the Dendrochirotida. We know nothing of the development in these sea cucumbers, but would expect similarities to their close relatives, the dendrochirotids. Egg sizes of 350 um have been reported for Cherbonniera utriculus (Tyler et al., 1985a) and Ypsilothuria talismani (Tyler and Gage, 1983); Tyler and Gage (1983) thought it likely that the latter dactylochirotid would have lecithotrophic development with a "reduced larval stage".

Aspidochirotida

It is in the order Aspidochirotida that the planktotrophic auricularia larval form is best known, due primarily to the investigations of Theodore Mortensen (1937, 1938) on 11 species in the Red Sea. The prevalent notion that the auricularia is the common larval form of sea cucumbers had its beginnings with Johannes Muller's first descriptions (1848 - 1853) of the holothuroid "pseudembryo" who gave it the name *auricularia*, because of the likeness that the lateral ciliary band convolutions have with the shape of the human ear. The success which early workers had in obtaining auricularia larvae, the transparency of the larva which permitted observation of internal developmental events, and the controversies concerned with the development and metamorphosis of the auricularia (Semon, 1888; Bury, 1889, 1895; MacBride, 1914), all served to focus attention on this larval form.

Of the approximately 292 species of aspidochirotids (Pawson, 1982), there is some information for the development of 25 species (Table VI-2). Ninety two percent (23 species) of these possess an auricularia and belong to the families Holothuriidae and Stichopodidae. These two families are distributed in shallow water in tropical and subtropical latitudes; exceptions are three stichopodids (Parastichopus californicus, Stichopus japonicus, and S. tremulus) that inhabit colder temperate waters. Some members of the Holothuriidae attain wide distributions (Table VI-2) and are spread from the Red Sea into the eastern Pacific (Holothuria difficilis, H. leucospilota, and H. pardalis) or can be circumtropical (H. arenicola and H. impatiens). Body lengths range from 6 - 90 cm and habitats include undersides of rock and coral, sandy bottoms, mud, sea grass beds, and reefs in breaking surf. Despite this variation in body size and habitat, these 23 holothuroids thus far seem to be fairly uniform in their early development: all of them produce an auricularia that varies little in appearance. Mortensen's (1921, 1937, 1938) larval identifications revealed that many auriculariae can only be distinguished by microscopical examination of the 4 - 93 um-long calcareous ossicles found in the posterior of the larva.

Owing to the fact that the critical period in development (where mortality is high) is the auricularia (Imai et al., 1950) and that Mortensen's studies (1921, 1937, 1938) concentrated on depicting the auricularia larvae in full form, later larval and juvenile stages

Table VI-2. Tabulation of adult size, egg size, habitat, and distribution for 25 species of the holothuroid order Aspidochirotida with known modes of development. Dashes indicate no data available and c.r. stands for ciliary rings.

SPECIES	MAXIMUM ADULT LENGTH (cm)	EGG SIZE (um)	DEVELOPMENTAL MODE	HABITAT, DISTRIBUTION	SOURCES
Actinopyga mauritiana	30	---	auricularia	on reefs where surf breaks, surge channels; Red Sea, Indo-Pacific	Mortensen, 1937; Lawrence, 1979; Sachithananthan et al., 1979
A. serratidens	15	---	auricularia, doliolaria (5 c.r.)	grass bottom; Red Sea	Mortensen, 1937; Cherbonnier, 1955
Bathyplotes natans	20	400-1100	eggs stuck to tentacles withdrawn into buccal cavity	200-1600 m; No. Atlantic, Caribbean, Japan, N. Zealand	Ohshima, 1915; Deichmann, 1930, 1954; Pawson, 1970; Lonning, 1976
Bohadschia marmorata	40^+	---	auricularia	coral sand, grass bottom; E. Africa, Red Sea, Indo-West Pacific	Mortensen, 1937; Sloan et al., 1979; Sachithananthan et al., 1979
Holothuria arenicola	50^+	--	auricularia, doliolaria (5 c.r.)	sand, sea grass beds, under rock; tropicopolitan	Clark, 1933; Mortensen, 1937; Macnae&Kalk, 1962;
H. difficilis	12	---	auricularia	sand, under rock, tide pools; Red Sea, east. & west. tropical Pacific	Deichmann, 1937; Mortensen, 1938; Rowe, 1969

H. floridana	20	200-400 (from hist-ological prepara-tions)	pelagic egg, hatches at pentactula	0.3-1 m, shallow bays; Caribbean (direct devel.)	Brooks, 1888; Edwards, 1889, 1907, 1908-1909, 1909; Engstrom, 1980a, b
H. forskali	25	---	auricularia	rocky-sandy-algal bottoms; Mediter-ranean, Brit. Isles, Scandinavia	Mortensen, 1927; Kaestner, 1963; Rowe, 1969; Gustato& Villari, 1979
H. glaberrima	20	90-131	auricularia	rocks, coral in surf; Caribbean	Clark, 1933; Lewis, 1960; F.S. McEuen, unpublished data
H. impatiens	40	---	auricularia, doliolaria (5 c.r.)	under rocks, sandy shores; tropicopolitan	Clark, 1933; Mortensen, 1938; Sloan et al., 1979
H. kefersteini	---	---	auricularia	rocky shores; Bay of Panama	Mortensen, 1921
H. leucospilota	60	141	auricularia	sand, under rock, intertidal reef flat pools; east. & west. Pacific, Red Sea	Endean, 1957; Bonham&Held, 1963; James&Pearse, 1969; Maruyama, 1980; Cherbonnier &Feral, 1984
H. nigra	---	---	auricularia	Gr. Britain	Mortensen, 1913
H. papillifera	---	100	auricularia	Red Sea	Mortensen, 1938
H. pardalis	10.7	124	auricularia	sand, gravel, under stones; east. & west. Pacific, Red Sea	Mortensen, 1938; Cherbonnier, 1955; James&Pearse, 1969; Sloan et al., 1979; Maruyama, 1980
H. poli	8.7	200	auricularia	sand; Mediter-ranean, Red Sea	Horstadius, 1925, 1939; Cherbonnier, 1955; James &Pearse, 1969
H. scabra	40	---	auricularia	sand, grass bottom; Red Sea, Indo-West Pacific	Mortensen, 1937; Sachithananthan et al., 1979
H. spinifera	24	---	auricularia	Red Sea	Mortensen, 1937; Clark, 1946

H. tubulosa	30	---	auricularia	rocky-sandy-algal bottoms;Mediterranean	Selenka,1876a,b;Kaestner,1963;Gustato&Villari,1979
Holothuria n. sp.(?)	6	---	auricularia	littoral;Gulf of Panama	Mortensen,1921
Microthele nobilis	56	140-160	auricularia	sand by coral heads,lagoons;Red Sea,Indo-West Pacific	Mortensen,1938;Sachithananthan et al.,1979;Conand,1981
Parastichopus californicus	78	185-210	auricularia, doliolaria (5 c.r.)	subtidal cobble,boulders;British Columbia to Lower California	Mortensen,1921;Clark,1922;Deichmann,1937;M. Strathmann,1974;R.R. Strathmann,1978;Smiley,1984;this study
Stichopus japonicus	43	150-180	auricularia, doliolaria (5 c.r.)	rocky,sandy,muddy bottoms;Japan,Korea	Clark,1922;Inaba,1937;Kinosita,1938;Imai et al.,1950;Tanaka,1958;Choe,1963;Suguri,1965
S. tremulus	50	150-154	auricularia	mud,20-1918m;North Sea,Norway,Sweden	Mortensen,1927;Rustad,1938;Jespersen&Lutzen,1971;Hansen,1975;Lonning,1976;Holland,1981
S. variegatus	90	---	auricularia; doliolaria (5 c.r.)	sand,turtle grass beds,coral rubble;Red Sea,Indo-West Pacific	Clark,1922,1946;Mortensen,1937;Sachithananthan et al.,1979;Sloan&von Bodungen,1980

have been followed for no more than six species. In all six of these, metamorphosis of the auricularia results in a non-feeding doliolaria with five ciliary rings. Larval metamorphosis is completed with emergence of five primary tentacles, settlement of the pentactula, and loss of external ciliation.

Associated with the uniformity of morphology among auricularia larvae is a narrow interval of small egg diameters of 90 - 210 um. There are two exceptions in the order, Holothuria floridana (200 - 400 um egg) and Bathyplotes natans (400 - 1100 um egg), both of which display different types of development. In a preliminary note on the early stages of H. floridana in the Bahamas, Edwards (1889, and in Brooks, 1888) stated that this species "follows a very complex development within the egg-shell" and "to all appearances the embryo passes through the auricularia stage", with a pentactula hatching at 5 d. Later statements in more complete accounts (Edwards, 1907, 1908-1909, 1909) of the development of H. floridana did not mention the presence of an auricularia enclosed within a vitelline membrane, but did acknowledge a spinning ciliated embryo surrounded by the egg membrane. Though Edwards' study is not conclusive, H. floridana does resemble Sclerodactyla briareus (a dendrochirotid) that also free-spawns eggs that develop directly and hatch as pentactulae (Ohshima, 1925).

The other anomaly among aspidochirotids is the discovery of eggs attached to the tentacles of a male Bathyplotes natans by Ohshima (1915). Provided with only this limited information, it is difficult to state with certainty that this is truly a case of brooding. The large egg size, the bathyal distribution of this form, as well as large eggs in other species of synallactid aspidochirotids (Table VI-3), though, would seem to support Ohshima's assumption that this species practices broodcare. Because of the brevity and incompleteness of the observations of Edwards and Ohshima on the above two species and the supposed striking contrasts these sea cucumbers might offer in their manner of brooding and course of development, it is hard to compare them to other aspidochirotids or with holothuroids in general.

Elasipodida

The difficulties involved with studying deep-sea fauna are manifest in the paucity of information on the biology of elasipodids, a strictly deep-sea group of holothuroids.

Table VI-3. Holothuroid egg sizes for species in which mode of development is not known. See Table VI-4 for elasipodid egg sizes.

SPECIES	EGG SIZE (um)	SOURCE
Dendrochirotida		
Athyonidium chilensis	500	Pawson, 1969
Cucumaria farquhari	1000+	Mortensen, 1925
C. hyndmanni	300	Lonning, 1976
C. japonica	500-540	Kinosita & Sibuya, 1941; Inaba, 1957
Ekkentropelma brychia	200	Pawson, 1971a
Heterothyone alba	200-300	Mortensen, 1925; Pawson, 1963
Lissothuria mortenseni	"less than 0.5 mm"	Pawson, 1967
Ocnus diomedeae	700	Pawson, 1976
Psolidiella nigra	1400	Pawson, 1970
Psolus neozelanicus	200	Mortensen, 1925
Trachythyone macphersonae	200-800	Pawson, 1968; Simpson, 1982
Dactylochirotida		
Cherbonniera utriculus	350	Tyler et al., 1985a
Ypsilothuria talismani	350	Tyler & Gage, 1983
Aspidochirotida		
Actinopyga echinites	175	Conand, 1982
Holothuria atra	130-140	Colwin & Colwin, 1955
H. thomasi	117	Pawson & Caycedo, 1980
Mesothuria intestinalis	500+	Theel, 1901; Lonning, 1976
Microthele fuscogilva	170	Conand, 1981
Paelopatides sp.	1440	Hufford, 1968
Pseudostichopus dilatorbis	226-444	Imaoka, 1978

Scotothuria sp.	1800	Billett & Hansen, 1985
Stichopus chloronotus	112	Sivickis & Domantay, 1928
Thelenota ananas	200	Conand, 1981
Apodida		
Chiridota carnleyensis	200	Mortensen, 1925
C. gigas	80	Mortensen, 1925
C. laevis	420	Heding, 1935
C. pisanii	300	Pawson, 1964
Kolostoneura novae-zealandiae	100	Mortensen, 1925
Leptosynapta transgressor	272	Brooks, 1973
Opheodesoma spectabilis	100	Berrill, 1966
Protankyra uncinata	100	Mortensen, 1925
Rhabdomolgus ruber	250	Menker, 1970
Molpadiida		
Caudina arenata	190-260	Gerould, 1896; Clark, 1907
Eupyrgus scaber	250	Heding, 1935
Molpadia antarctica	300	Pawson, 1969

The extent of our knowledge on elasipodid reproduction is for the most part restricted to egg sizes. If the commonly accepted idea that the incidence of non-pelagic development in marine invertebrates increases with larger egg size and increasing depth (Thorson, 1936, 1950) is true, one would predict the majority of elasipodids to practice some form of broodcare, from the extraordinarily large diameters of their ovarian oocytes (Table VI-4). But notwithstanding the extensive collections of deep-sea expeditions and the detailed taxonomic work of Theel (1882, 1886) and particularly of Hansen (1975) on the Elasipodida, we know of only one instance of brooding.

Oneirophanta mutabilis was discovered by Hansen (1968) to contain young up to 30 mm long in the ovaries. He proposed that in earlier stages, young may feed on unfertilized eggs and that more developed stages are nourished by a nutritive fluid secreted into the ovaries. His later study (Hansen, 1975) of gonad histology of this same species showed highly vacuolated cells in the ovarian wall that develop shortly after spawning and collapse with growth of the young. He suggested this vacuolated tissue acted as a store which provides nutrients for the growing embryos. The difference in juvenile tentacle disc morphology from that of the adult (the juvenile tentacle discs having papillae and incisions) was also interpreted as functioning in taking up nutrient matter secreted from the ovarian wall.

Apodida

The diversity of variations of basic holothuroid developmental patterns in the apodids is unequalled in the other five orders of holothuroids. I have categorized the main patterns of apodid development from the data in Table VI-5 as follows:

Pelagic development:

1) planktotrophic auricularia succeeded by a doliolaria with five ciliary rings, and
2) doliolaria with four ciliary rings.

Brooded development:

1) coelomic brooding (uniformly ciliated larva, five-ringed doliolaria, reduced auricularia followed by four-ringed doliolaria, or direct-developing young), and

Table VI-4. Listing of elasipodid holothuroids with reported egg sizes; arranged according to family.

SPECIES	EGG SIZE (um)	SOURCE
Deimatidae		
Deima validum	650-800	Hansen, 1975; Tyler et al., 1985a
Oneirophanta mutabilis mutabilis	900	Hansen, 1975
O. mutabilis affinis	200 (brooder)	Hansen, 1968, 1975
O. mutabilis	650	Tyler et al., 1985a
O. setigera	300	Hansen, 1975
Orphnurgus glaber	300	Hansen, 1975
Laetmogonidae		
Benthogone rosea	650-900	Hansen, 1975; Tyler et al., 1985a,b
Laetmogone fimbriata	190	Hansen, 1975
L. maculata	150	Hansen, 1975
L. violacea	350-400	Hansen, 1975; Tyler et al., 1985a,b
Pannychia moseleyi	800	Hansen, 1975
Psychropotidae		
Benthodytes incerta	1700-2500	Ohshima, 1915; Hansen, 1975
B. lingua	2000	Hansen, 1975
B. sanguinolenta	2000-2200	Ohshima, 1915; Hansen, 1975
B. sibogae	900-1900	Pawson, 1965; Hansen, 1975
B. typica	3000	Hansen, 1975
Psycheotrephes exigua	3700	Hansen, 1975
Psychropotes aff. minuta	1000	Madsen, 1947-1948
P. belyaevi	3600	Hansen, 1975

P. depressa	1900-2500	Ohshima, 1915;Hansen, 1975
P. longicauda	4400-4500	Hansen, 1975;Billett& Hansen, 1985
P. semperiana	500	Hansen, 1975
P. verrucosa	3000	Hansen, 1975
Elpidiidae		
Amperima naresi	200	Hansen, 1975
Elpidia glacialis	200-280	Thorson, 1936;Hansen, 1975
Kolga hyalina	180	Billett&Hansen, 1982
Peniagone azorica	200	Hansen, 1975
Peniagone sp.	350	Tyler et al., 1985a
Scotoplanes clarki	200	Hansen, 1975
S. globosa	200	Hansen, 1975
Pelagothuriidae		
Enypniastes eximia	3000-3500	Ohshima, 1915
Enypniastes sp.	3500	Billett&Hansen, 1985
Pelagothuria natatrix	1200	Ludwig, 1894(from Mortensen, 1927)

Table VI-5. Tabulation of adult size, egg size, habitat, and distribution for 15 species of the holothuroid order Apodida with known modes of development. Dashes indicate no data available; c.r. is for ciliary rings.

SPECIES	MAXIMUM ADULT LENGTH (cm)	EGG SIZE (um)	DEVELOPMENTAL MODE	HABITAT, DISTRIBUTION	SOURCES
Chiridota rotifera	10	---	broods reduced auricularia, doliolaria(4 c.r.) in coelom	sand, sea grass beds, under stones; intertidal; Bermuda to Brazil	Ludwig, 1880, 1881; Clark, 1899, 1907, 1910; Engstrom, 1980c
Epitomapta roseola	10	---	free-swimming larva	sand, mud, under rocks, shallow; Massachusetts, Connecticut, Bermuda	Clark, 1899, 1907; Coe, 1912
Labidoplax buskii	3	150	doliolaria (4 c.r.)	mud, 18-445 m; no. European coasts, No. Carolina	Clark, 1907; Mortensen, 1927; Nyholm, 1951; Pawson, 1967
L. digitata	35	---	auricularia, doliolaria (5 c.r.)	muddy-sandy bottom; Mediterranean, Gr. Britain	Metschnikoff, 1869; Selenka, 1876, 1883; Semon, 1888; Bury, 1889, 1895; Clark, 1907; Reimers, 1912; Chadwick, 1914; Mortensen, 1927
Leptosynapta clarki	12	240-404	ovarian brooder, hatch as pentactulae	muddy sand, *Zostera* beds, bays; Queen Charlotte Is. to central California	Heding, 1928; Everingham, 1961; Brooks, 1973; this study
L. gallienei	30	---	doliolaria (4 c.r.)	muddy sand; no. European coast	Clark, 1907; Cherbonnier, 1953

L. inhaerens	30	200	doliolaria (4 c.r.)	mud,sand,*Zostera* banks;circumpolar	Clark,1907;Runnstrom,1924, 1927;Mortensen&Lieberkind, 1928;Cherbonnier,1953
L. minuta	1	---	broods in coelom (direct devel.)	mud,sand(interstitial);North Sea,Helgoland, France	Becher,1906;Mortensen, 1927;Cherbonnier,1953; Swedmark,1964
L. tenuis	18	200-209	pelagic larva	mud,sand,*Zostera* beds, intertidal; New England coast	Clark,1902b;Costello et al.,1957;McCrary,1969; Green,1976,1977,1978
Opheodesoma grisea	90	100	auricularia	Red Sea,Indo-West Pacific	Clark,1907;Mortensen, 1938
Synaptula hydriformis	15	210	broods uniformly ciliated larvae in coelom	seaweed on mangrove roots;Bermuda to Brazil	Oerstedt,1849-1850;Ludwig, 1881,1886;Clark,1896a, b,1898;Clark,1907;Turner &Dearborn,1979
S. reciprocans	---	50	auricularia	loose rock& coral,phanerogam beds;Red Sea	Mortensen,1937;James & Pearse,1969
S. vittata	---	---	auricularia, doliolaria (5 c.r.)	under algae,rocks, coral,shore flats; Red Sea	Mortensen,1937,1938
Taeniogyrus contortus	4.5	330	broods in ovaries	sand,mud,10-220 m;Antarctic	Ludwig,1897,1898,1904; Clark,1902b,1907
Trochodota dunedinensis	5	200	broods doliolaria(5 c.r.) in coelom	littoral;New Zealand	Dendy,1898;Mortensen, 1925;John,1939

2) ovarian brooding (direct development).

Free-swimming larvae (outside the female) are known for nine apodids (Table VI-5); four of these have an auricularia larva (Labidoplax digitata, Opheodesoma grisea, Synaptula reciprocans, and S. vittata), two of which metamorphose into a five-ringed doliolaria, similar to aspidochirotids. Although the number of species in this category of planktotrophic larvae is small, the available data show eggs to be small (50 - 100 um) and adults to be very large (35 - 90 cm long) and distributed in warm waters (Indo-West Pacific, Red Sea, and Mediterranean). Labidoplax digitata is also encountered in the British Isles, but is primarily Mediterranean in its distribution.

In the colder waters of northern Europe in mud and sand, three apodids (Labidoplax buskii, Leptosynapta gallienei, and L. inhaerens) of smaller body size (3 - 30 cm) release 150 - 200 um eggs that develop into doliolaria larvae with four ciliary rings, bypassing the auricularia. Leptosynapta inhaerens is considered to be circumpolar (Clark, 1907), but many species have probably been confused under this name (Deichmann, 1930). Leptosynapta tenuis, from indications of its egg size, body length, habitat, and distribution in temperate latitudes, may also prove to have a four-ringed doliolaria.

Coelomic brooding apodids present some very unusual departures from the direct development that is so common among the numerous brooding dendrochirotids (chapters four and five). Three of these small, thin-skinned sea cucumbers incubate various larval types within the perivisceral coelom, including uniformly ciliated larvae (Synaptula hydriformis), doliolariae with five ciliary rings (Trochodota dunedinensis), or reduced (modified) auriculariae and four-ringed doliolariae (Chiridota rotifera). This last species possesses the only form known that might be considered intermediate between the auricularia and doliolaria. Clark's description (1910) of ovarian brooding of larvae of C. rotifera is brief, but he does describe and depict in his illustrations a dolioform larva lacking a mouth and wearing lateral ciliary bands; the latter are clearly unlike any of the transverse rings worn by any holothuroid doliolaria. These three larval incubating species can be found in very shallow water (intertidal or on mangrove roots), two of them in the Caribbean and one in New Zealand. It is common knowledge that apodids possess anal pores (Clark, 1898; Becher, 1912; Anderson, 1966; Turner, 1973), creating the possibility of larvipary among these larval brooders.

The fourth species practicing coelomic broodcare is Leptosynapta minuta, a north European synaptid of only 4 - 10 mm length. Typical of interstitial fauna, this smallest of sea cucumbers broods a small number of embryos (maximum of five); no stages other than pentactulae have been found and it is assumed development is direct.

The last grouping of apodids with similar development is composed of two ovarian brooders from cold waters (temperate north Pacific and Antarctic), Leptosynapta clarki and Taeniogyrus contortus, respectively. Ludwig (1897, 1898) recorded pentactulae, juveniles with seven tentacles, and also eggs occurring in the same ovary in individuals of T. contortus. Everingham (1961) followed the development of L. clarki, noting this synaptid to pass through a non-ciliated gastrula stage and hatching as a pentactula within the ovary. Unfertilized eggs, termed "inert cells" by Everingham, accompany embryos in the ovary throughout development. Everingham suggested these eggs might provide nutrition to pentactulae and later stages; the largest young he encountered in ovaries were 6.3 mm long with 12 tentacles. My observations on L. clarki development confirm Everingham's suggestion: pentactulae and older stages do consume nurse eggs. Whole eggs have been seen in the guts of the young. In some females, these nurse eggs in the ovaries break up into small granules; the granules, too, were observed in the stomachs of developing young. In late October, when breeding aggregations occur in False Bay, San Juan Island (Everingham, 1961), fecundity counts were taken in 1982 for 11 ripe L. clarki females. Fecundity ranged from 409 - 2,495 eggs with a mean fecundity of 1124 ± 607 eggs/female. In late March and April when young are released (Everingham, 1961), eight females were collected on 10 April and eggs and juveniles in ovaries were enumerated. Mean number of juveniles being brooded in ovaries just prior to release was 44 ± 39 juveniles/female and mean number of remaining eggs in the ovaries was 46 ± 102 eggs/female. In April, the large juveniles fit snugly in the ovarian tubules, frequently lined up lengthwise in single file and filling the tubule. The largest ovarian juvenile I measured was 12 mm long.

Included in this section of the Apodida are the unusually large auricularia larvae that measure 3 - 15 mm in length. Collected from both the Atlantic and Pacific Oceans, these larvae are covered with complex arabesque-like folds of the ciliary band and have been variously named Auricularia nudibranchiata, A. plicata, A. antarctica, and A. bermudensis (reviewed by Pawson, 1971c). There remains the question as to which species they belong, but from the characters of the larval calcareous wheels, it has been concluded that they are synaptids. Young pelagic synaptid juveniles with reduced, but elaborate ciliary banding, were caught along with A. nudibranchiata by Inaba (1934): the juveniles and larvae contained wheels of the same type and size. Teleplanic larvae are larvae of benthic continental shelf species that disperse long distances (Scheltema, 1964, 1971) and exhibit elaborate adaptations for their protracted pelagic life; the gigantic synaptid auriculariae may well prove to belong to this group of larvae which migrate long distances.

In summary, the order Apodida has distinct modes of development, each with characteristic egg sizes; some of them also have separate distributions and body sizes. Apodid genera are not limited to a single mode of development: species of Synaptula are either brooders or are oviparous with auricularia larvae, and in Leptosynapta, coelomic and ovarian brooding and pelagic doliolaria larvae have been described. Overall, adults of species producing free-swimming larvae are 3 - 90 cm in length and have eggs of 50 - 209 um diameter, whereas brooders are 1 - 15 cm long with 200 - 404 um eggs.

Molpadiida

A single larval type, a yolky doliolaria with two ciliary rings, belongs to this cosmopolitan group of mud and sand dwellers. Knowledge of reproduction in this taxon (treated in chapter three) is based on the two bathyal and abyssal forms, Molpadia intermedia and Paracaudina chilensis. Egg diameters are 267 and 572 um, respectively.

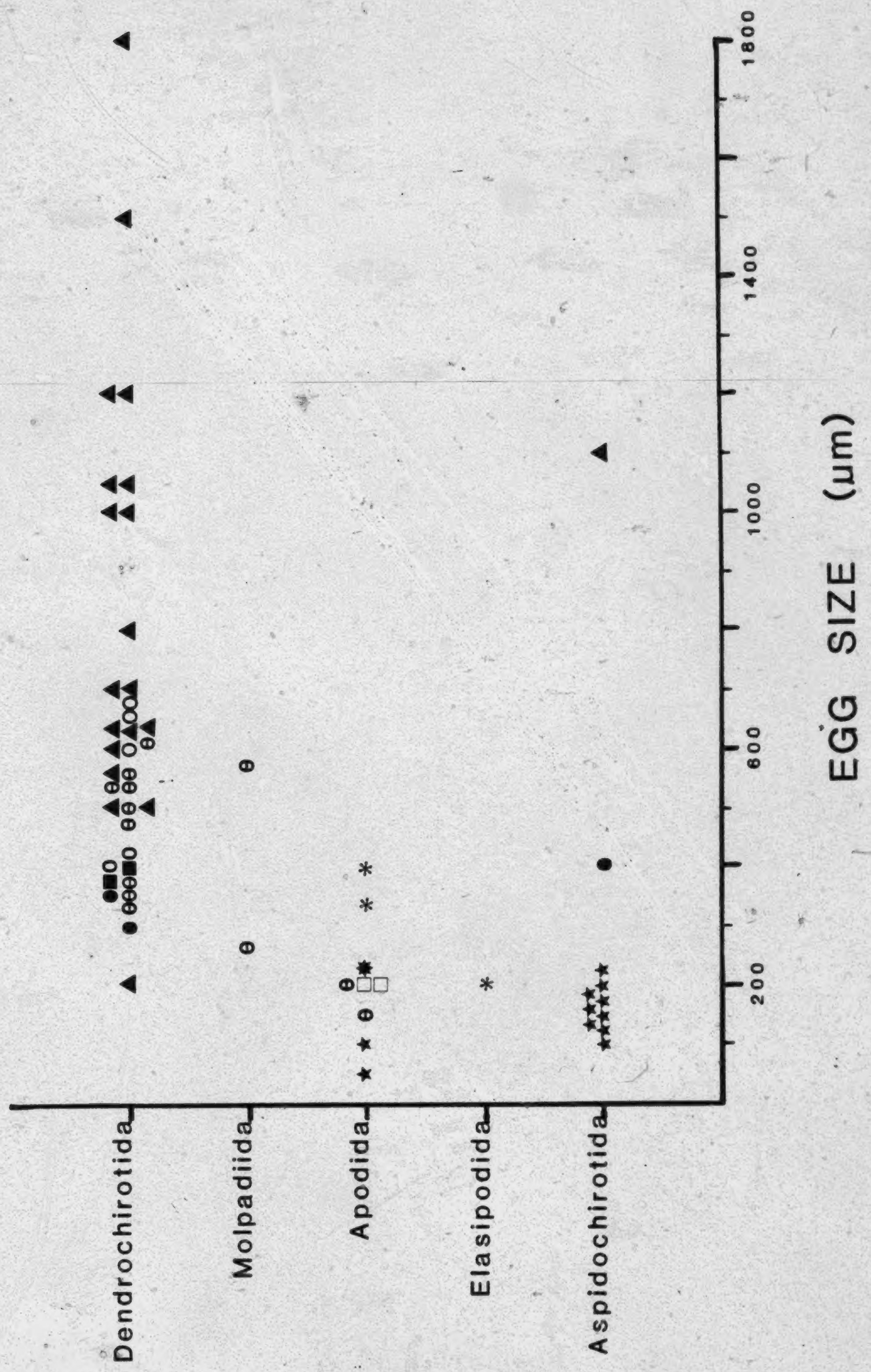
EARLY LIFE HISTORY CHARACTERISTICS

Egg Size

Egg diameter has been a traditional measurement used for predicting developmental mode. In the Holothuroidea, egg size can sometimes be a useful indicator, depending on the taxon being considered.

The dendrochirotids exhibit the following egg diameters with these patterns of development (Fig. VI-3): evenly ciliated larvae or doliolaria with ciliary rings, 330 - 670 um; pelagic egg (with direct development), 300 - 350 um; and external or coelomic brooding,

Fig. VI-3. Distributions of known developmental types for five of the six holothuroid orders according to egg size. Symbols: star (five-rayed) - auricularia, lined circle - doliolaria, open circle - evenly ciliated larva, solid circle - pelagic direct development, triangle - external brooder, star (eight-rayed) - unspecified pelagic larva, open square - coelomic brooder (larvae), solid square - coelomic brooder (direct development), asterisk - ovarian brooder.


Dendrochirotida
Molpadiida
Apodida
Elasipodida
Aspidochirotida
200
600
1000
1400
1800
EGG SIZE (µm)

200 - 1800 um. Even with the free-swimming larvae having an approximately 700 um upper limit in egg size, there is considerable overlap in egg size with brooding species, making predictions on development solely from egg diameters in this order of limited value.

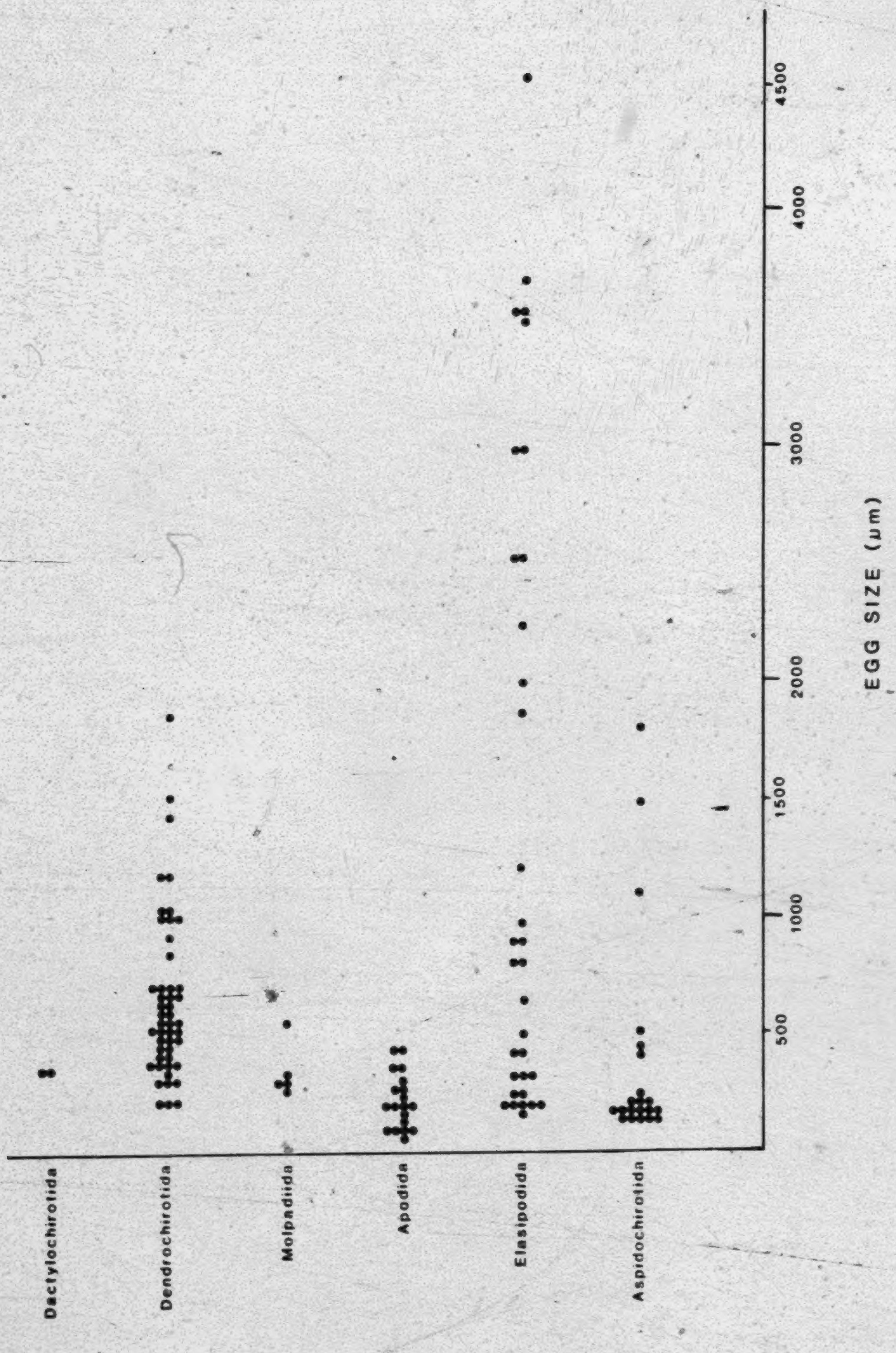
The nine apodids show a mixed bag of developmental patterns, with seven of them having a 50 - 209 um egg diameter range. Although the number of species with known development is small, apodids with auricularia larvae have the smallest eggs (50 - 100 um) and are distinct from forms with doliolaria larvae (150 - 200 um eggs) and coelomic brooding (200 um eggs); ovarian brooders are set apart with their large eggs (340 - 404 um).

Auricularia larvae, all developing from eggs smaller than 210 um, characterize the two aspidochirotid families Holothuriidae and Stichopodidae. A single holothuriid, Holothuria floridana, with an egg of 400 um, presents the sole exception; the information on development and egg size of this aspidochirotid, though, is not clear and remains tentative (Brooks, 1888; Edwards, 1889, 1907, 1908-1909, 1909; Engstrom, 1980a,b). Brooding (among the tentacles) is the other aspidochirotid developmental mode and is found in a deep-sea synallactid.

The extremely broad range of holothuroid egg diameters (Fig. VI-4, which includes egg sizes from Table VI-3) encompasses almost two orders of magnitude from 50 um (Synaptula reciprocans; Mortensen, 1937) to 4500 um (Psychropotes longicauda; Billett and Hansen, 1985). In comparing Figure VI-4 with Figure VI-3, additional reported egg sizes included in Figure VI-4 seem to reinforce the distribution of egg sizes where development is known, inferring that the existing developmental patterns are representative of the various taxa (but see below for Elasipodida). The five largest eggs in the Aspidochirotida belong to the Synallactidae, a deep-sea family isolated from the two shallow-water families Holothuriidae and Stichopodidae.

The extraordinary size and spread of elasipodid egg diameters (Fig. VI-4) are traits that distinguish this group from all other echinoderms. Hansen (1975) pointed out conspicuous differences in egg size among benthic elasipodid families and indicated there is a positive interspecific correlation between increasing egg size and adult depth occurrence in the Laetmogonidae and Psychropotidae. Since the known development in

Fig. VI-4. Distributions of reported maximum egg sizes of holothuroids, grouped by order.

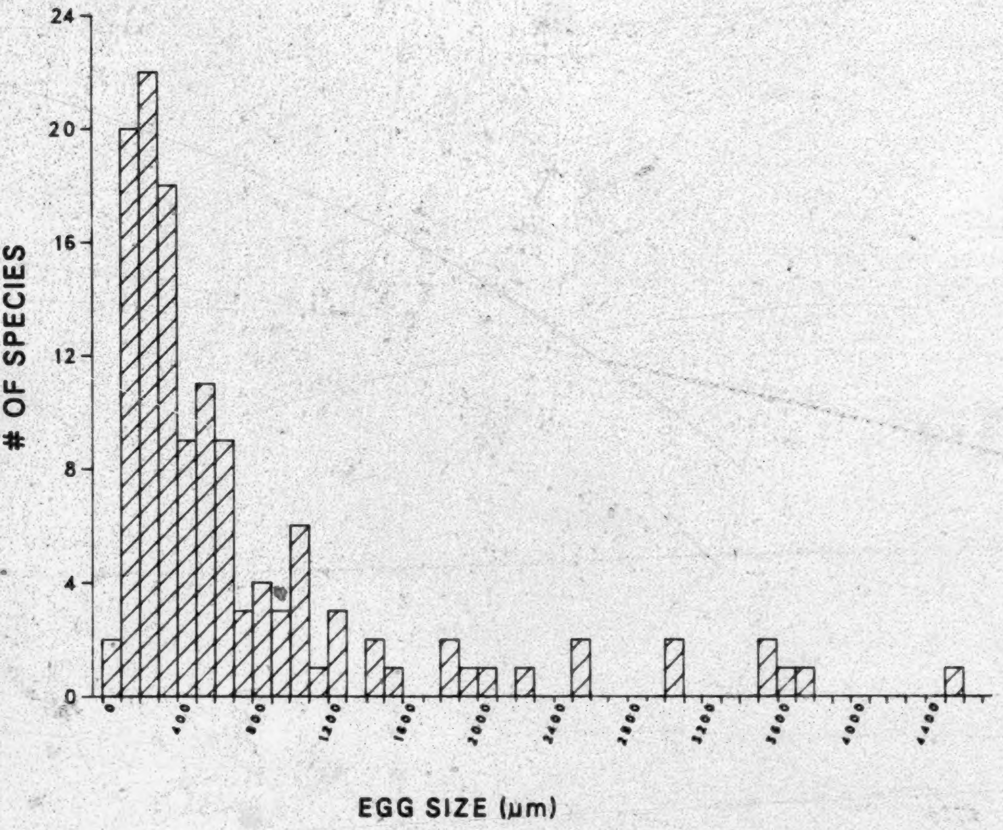

Dactylochirotida
Dendrochirotida
Molpadiida
Apodida
Elasipodida
Aspidochirotida
500
1000
1500
2000
3000
4000
4500
EGG SIZE (µm)

this order is restricted to a single case of brooding. Hansen (1975) used egg sizes and proposed that there are different types of larval development in the Laetmogonidae (150 - 900 um eggs; Table VI-4) and lecithotrophic development in the Elpidiidae (180 - 350 um eggs). He also emphasized the 500 - 4500 um eggs in the abyssal Psychropotidae. Juveniles in this family were taken in a pelagic net which suggested to Hansen (1975) that "the enormous egg size ... is an adaptation which ensures a long pelagic development independent of food uptake from the plankton -- thus an adaptation to life in the nutrient-poor deep sea." Hansen's statement appears supported by the 1200 - 3500 um egg size range in the Pelagothuriidae, a family of swimming forms that spend most of their time off the sea floor. In summarizing his egg size data, Hansen's opinion was that even the smallest elasipodid eggs probably produced lecithotrophic larvae.

Looking at the class as a whole, the mean egg diameter of 716 um is reduced to 447 when the Elasipodida and Synallactidae (the deep-sea taxa) are excluded. Contrary to theoretical predictions (Vance, 1973), the distribution of holothuroid egg sizes does not display a bimodal pattern for the entire class (Fig. VI-5) nor in the orders (Fig. VI-4), with the possible exception of the Apodida. Mean egg sizes for the six orders of holothuroids are: Dendrochirotida, 647 um; Dactylochirotida, 350 um; Aspidochirotida, 381 um (170 um without the Synallactidae); Elasipodida, 1388 um; Apodida, 204 um; and Molpadiida, 330 um. There are family-specific egg size ranges in the Elasipodida and Aspidochirotida as discussed above and, in the latter order, egg diameter is of predictive value for developmental mode. It is not presently possible, however, to talk of familial egg characteristics in the Dendrochirotida since many taxa within this group require systematic revision (D. Pawson and M. Bergen, personal communication). Neither can strict egg size categories corresponding to particular patterns of development be constructed. As examples, intra-ordinal overlap in egg size occurs with lecithotrophic larvae and brooders in the Dendrochirotida and also in the Apodida. Interordinally, the small lecithotrophic larvae of the apodids overlap in their egg diameters with those of aspidochirotid auriculariae. It can be said, though, in a general way, that eggs smaller than 200 - 210 um will develop into a planktotrophic auricularia in the Aspidochirotida and Apodida and that eggs larger than this in all taxa possess either a lecithotrophic larvae, pelagic direct development, or some form of external / internal broodcare. Among brooders, species

Fig. VI-5. Combined holothuroid egg sizes exhibiting a unimodal distribution; *n* = 128.

Distribution of Holothuroid Egg Sizes



with developing young in the ovaries or larvae in the coelom produce eggs varying 200 - 404 um in diameter, whereas 19 of 20 externally brooding species have 500 - 1800 um eggs (the one exception is the 200 um egg of *Trachythyone parva*; see Table V-3).

Fecundity

The extremes in number of eggs produced by sea cucumbers are two in *Leptosynapta minuta* and 8.92 x 10^6 in *Parastichopus californicus* (Table VI-6). Assuming that brooded eggs, embryos, or young are indicators of ovarian fecundity, species with broodcare lay a maximum of 5 - 522 eggs. The one ovarian brooder for which egg number has been counted is *Leptosynapta clarki*, which holds 409 - 2,495 eggs prior to fertilization that takes place in the ovaries. Since pentactulae and later stages of this synaptid consume nurse eggs, juvenile number is not a reliable indicator of egg number. The next category has maximum fecundities of 3,074 - 3.592 x 10^5 eggs and comprises those holothuroids with non-feeding larvae. High egg numbers distinguish three aspidochirotids and an apodid with auricularia larvae: 5 x 10^5 - 8.92 x 10^6 are maximum fecundities for these large forms.

The measured fecundities fall into convenient groups that correspond with different developmental modes (Fig. VI-6). Means of the fecundities for the three modes of development are significantly different ($p < 0.01$, RANOVA, SPSSX, Version 2). However, the high fecundities (from species with feeding larvae) belong to very large holothuroids 43 - 200 cm long (Tables VI-2 and VI-5; Berrill, 1966), and no fecundity estimates are yet available for the smaller tropical aspidochirotids whose egg numbers may overlap the highest fecundities of lecithotrophic species. Direct egg counts for elasipodids have not been made, either, but would be expected to be quite variable in view of their wide range of egg sizes (Table VI-3).

Since most sea cucumbers have annual breeding seasons, the fecundities listed in Table VI-6 are presumed to be yearly fecundities. No attempts at calculating the total number of eggs laid by an individual in its lifetime have been made because no accurate measurements exist for holothuroid lifespans.

Table VI-6. Holothuroid fecundity. In parentheses, fecundity is specified as either brooded young, or eggs (brooded, ovarian, or spawned).

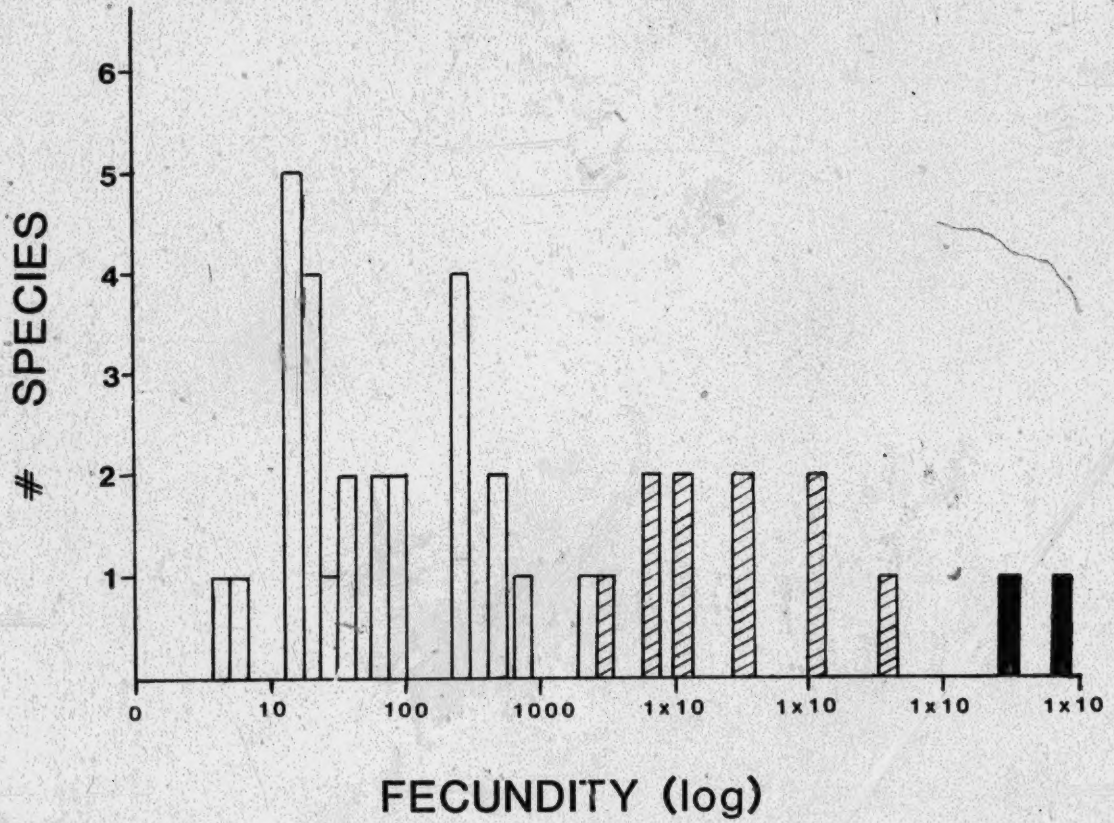
SPECIES	FECUNDITY	SOURCE
Dendrochirotida		
Cladodactyla crocea	140 (embryos in pouches)	MacBride&Simpson, 1908
Cucumaria frondosa	1,000 - 10,000 (spawned eggs?)	Falk-Petersen, 1982b
C. ijimai	19 (young in pouches)	Ohshima, 1915
C. lamperti	29 - 43 (young in pouches)	Ohshima, 1915
C. lubrica	152 (brooded young)	Engstrom, 1982
" "	61 - 372 (ovarian eggs)	this study
C. miniata	4,900 - 131,800 (spawned eggs)	this study
C. piperata	5,200 - 10,800 (spawned eggs)	this study
C. pseudocurata	340 (ovarian eggs)	Rutherford, 1973
" "	73 - 229 (brooded young)	Turner&Rutherford, 1976b
" "	22 - 149 (ovarian eggs)	this study
Cucumaria sp.	3,700 - 8,800 (spawned eggs)	this study
Eupentacta quinquesemita	29,900 - 105,100 (spawned eggs)	this study
Leptopentacta elongata	8,000 (ovarian eggs)	Chia&Buchanan, 1969
Lissothuria antillensis	80 - 90 (young in coelom)	Miller, 1983, 1985
L. nutriens	160 (spawned eggs)	Wootton, 1949

Neoamphicyclus lividus	23 (young in coelom)	Hickman, 1978
Neocnus incubans	30+ (eggs in pouch)	Cherbonnier, 1972
Ocnus sacculus	19 (embryos in sacs)	Pawson, 1983
Pachythyone lugubris	13 (embryos in coelom)	Deichmann, 1939
P. rubra	10 (young in coelom)	Clark, 1901
Pentamera populifera	103 - 35,300 (spawned eggs)	this study
Pseudocnus laevigatus	12 (eggs in pouches)	Pawson, 1971b
" "	93 (young in pouches)	Simpson, 1982
Pseudocucumis africanus	25 - 27 (brood in coelom)	Ohshima, 1916
Pseudopsolus macquariensis	14 (ovarian eggs)	Mortensen, 1925
" "	73 - 154 (eggs in sacs)	Simpson, 1982
Psolidium bullatum	90 - 3,074 (spawned eggs)	this study
P. incubans	22 (eggs on sole)	Ekman, 1925
Psolus antarcticus	22 (young on sole)	Ludwig, 1898
P. chitonoides	2,200 - 34,700 (spawned eggs)	this study
P. figulus	40 (eggs on sole)	Ekman, 1925
P. punctatus	40 (eggs on sole)	Ekman, 1925
Thyone imbricata	11 (young in pouches)	Ohshima, 1915
Trachythyone macphersonae	110 - 130 (ovarian eggs)	Simpson, 1982
T. parva	70 (attached young)	Ludwig, 1898

Dactylochirotida		
Ypsilothuria talismani	50 (ovarian eggs)	Tyler&Gage, 1983
Aspidochirotida		
Holothuria mexicana	466,000-884,000 (spawned eggs)	this study
Paelopatides sp.	127 (ovarian eggs)	Hufford, 1968
Parastichopus californicus	8,920,000 (ovarian eggs)	this study
Stichopus japonicus	183,000 - 263,000 eggs / gram of ovary wt.	Choe, 1963
" "	500,000 - 3,000,000 (spawned eggs)	Suguri, 1965
Elasipodida		
Oneirophanta mutabilis affinis	1 - 6 (young in ovaries)	Hansen, 1968
Apodida		
Chiridota rotifera	522 (young in coelom)	Clark, 1910
Leptosynapta clarki	409 - 2,495 (ovarian eggs)	this study
L. minuta	5 (juveniles in coelom)	Becher, 1906
" "	2 (juveniles in coelom)	Cherbonnier, 1953
" "	2 - 4 (embryos in coelom)	Swedmark, 1964
Opheodesoma spectabilis	500,000+ (eggs, estimated by gonad volume)	Berrill, 1966
Synaptula hydriformis	176 (young in coelom)	Clark, 1896a

Trochodota dunedinensis	27 - 75 (embryos / young in coelom)	John, 1939
Molpadiida		
Molpadia intermedia	293,800 - 359,200 (ovarian eggs)	this study

Fig. VI-6. Distribution of estimated fecundities for 38 holothuroid species with known modes of development. Open bars: brooders; hatched bars: pelagic lecithotrophic larvae; solid bars: pelagic planktotrophic larvae.


# SPECIES
6
5
4
3
2
1
0
10
100
1000
1x10
1x10
1x10
1x10
FECUNDITY (log)

Length of Pelagic Period and Size at Metamorphosis

Despite the well-known position the auricularia holds as the typical larva of holothuroids, the length of the pelagic period from fertilization to settlement is known only for Parastichopus californicus and Stichopus japonicus (Table VI-7). Knowledge of these two pelagic periods, in combination with other incomplete accounts, provide an estimate of the time planktotrophic holothuroid larvae spend in the plankton. The minimum time to settlement is 13 d (S. japonicus) and the longest is 133 d (in P. californicus; Table VI-7). Of the nine species of aspidochirotids and apodids which Mortensen (1937, 1938) cultured from the Red Sea, six reached the doliolaria stage in 2 - 5 wk and auriculariae of the other three species lived 14 d - 3 wk (from fertilization). The common pelagic periods, then, for cold temperate and tropical species with feeding larvae range from 2 - 8 1/2 wk.

Holothuroids with non-feeding larvae develop and settle in 4 - 13 d. Those having direct development within the egg membrane are in the plankton 3 1/2 - 6 d (Table VI-7).

The significance of the differences in length of the pelagic period in the above three groups is not yet understood. There are no data on the duration of holothuroid larval life in the field and only laboratory culture times are available. The short development times of pelagic eggs with direct development may be an adaptation to restrict dispersal in organisms living in estuaries and bays (Mileikovsky, 1971; Stancyk, 1973; Hendler, 1977). Dispersal over longer distances and growth have been associated with the longer-lived planktotrophic larvae (Thorson, 1946, 1950; Mileikovsky, 1971), but recent considerations of the supposed advantages attributed to the greater spread of larvae have doubted that the length of the pelagic period is determined by selection for dispersal (Strathmann, 1980; Palmer and Strathmann, 1981). The circumtropical distributions of Holothuria arenicola and H. impatiens and the wide distributions of other aspidochirotids from east Africa into the western Pacific attest to the dispersal capabilities of auriculariae (Bakus, 1973).

The larger juvenile size that results from planktotrophic larvae having an increased feeding time in the plankton is another benefit thought to enhance juvenile survival. A few species in the asteroid genus Luidia with planktotrophic larvae produce newly metamorphosed juveniles of 1 - 4 mm diameter (Wilson, 1978; Komatsu, et al., 1982;

Table VI-7. Length of pelagic period for free-swimming holothuroid larvae and pelagic eggs (with direct development), with corresponding egg size and the temperature at which embryonic and larval stages were reared. The pelagic period is the time interval from fertilization to settlement, unless stated otherwise. Three classifications of developmental type are employed to group the species: auricularia, doliolaria (with and without ciliary rings), and pelagic direct development (free-spawned eggs that develop directly in the plankton).

SPECIES	EGG SIZE	PELAGIC PERIOD	CULTURE TEMPERATURE (°C)	SOURCE
AURICULARIA				
Actinopyga serratidens	---	4 wk (to doliolaria)	---	Mortensen, 1937
Holothuria arenicola	---	4 wk (to doliolaria)	---	Mortensen, 1937
H. difficilis	---	2 wk (to doliolaria)	---	Mortensen, 1938
H. glaberrima	90-131	2 wk (to doliolaria)	---	Lewis, 1960
H. impatiens	---	3 wk (to doliolaria; some to 6 wk)	---	Mortensen, 1938
H. scabra	---	18 d (almost to doliolaria)	---	Mortensen, 1937
Opheodesoma grisea	100	14 d (still auriculariae)	---	Mortensen, 1938
Parastichopus californicus	---	51 d (91 d maximum)	7-14	Strathmann, 1978
" "	---	60 d (to doliolaria; 90 d maximum)	---	Cameron, 1981
" "	204	60-61 d (133 d maximum)	11	this study
" "	"	13-14 d (to doliolaria)	20-24	this study
Stichopus japonicus	---	15-23 d	20-22	Imai et al., 1950
" "	---	13-18 d	---	Suguri, 1965

" "	---	13-17 d	20-24	Oshima&Takeuchi, 1977
Stichopus tremulus	150	1 auricularia lived 1 1/2 months	---	Rustad, 1938
S. variegatus	---	35-40 d (to doliolaria)	---	Mortensen, 1937
Synaptula reciprocans	50	stayed alive 3 wk	---	Mortensen, 1937
S. vittata	---	2 wk (to doliolaria; some 4-5 wk)	---	Mortensen, 1938
DOLIOLARIA				
Aslia lefevrei	---	5 d	---	Newth, 1916
" "	400-650	5-7 d	---	Costelloe, personal communication
Cucumaria echinata	400-440	7 d	---	Ohshima, 1918, 1921
C. frondosa	500⁺	9 d	---	Jordan, 1972
C. miniata	520	8-13 d	11	chapter 5
C. piperata	532	8-9 1/2 d	11	chapter 5
Cucumaria sp.	504	9 1/2 - 10 3/4 d	11	chapter 5
Eupentacta quinquesemita	397	6 1/2 - 7 1/2 d	11	chapter 5
Labidoplax buskii	150	11-12 d	9	Nyholm, 1951
Leptopentacta elongata	250-350	9 d	9-11	Chia&Buchanan, 1969
Molpadia intermedia	267	95-96 hr	11	chapter 3
Ocnus planci	less than 500	doliolaria passed on 8th and 9th d	---	Ludwig, 1891
Paracaudina chilensis	572	9 d	13.7	Inaba, 1930
Pawsonia saxicola	---	5 d	---	Newth, 1916
Pentamera populifera	372	6 - 7 1/2 d	11	chapter 5

Psolidium bullatum	330	7 3/4 - 8 1/2 d	11	chapter 4
Psolus chitonoides	627	11-12 d	11	chapter 4
PELAGIC DIRECT DEVELOPMENT				
Holothuria floridana	200-400	5-6 d	---	Brooks, 1888; Edwards, 1889, 1907, 1908-1909, 1909; Engstrom, 1980a
Sclerodactyla briareus	250-300	3 1/2 d	---	Ohshima, 1925
Thyone fusus	350	4 d	---	Costelloe, personal communication

Domanski, 1984), but as yet there is no comparable example of auriculariae turning out large juveniles.

Data collected for 11 species of holothuroids from the San Juan Islands show a positive correlation ($r=0.932$, $p < 0.01$) between egg size and length at metamorphosis (Fig. VI-7). The smallest egg in Figure VI-7 belongs to the planktotrophic Parastichopus californicus; the two largest eggs are from the brooders Cucumaria lubrica and C. pseudocurata, and the eight intermediate-sized eggs belong to species with non-feeding larvae. Similar to P. californicus is Stichopus japonicus, another stichopodid with small eggs (150-180; Table VI-2), that is 0.3 -0.4 mm long 10 d after settlement (Inaba, 1957). Omitted from Figure VI-7 is Leptosynapta clarki whose oophagic young grow to a length of 12 mm in the ovaries. From the large sizes of brooded young recorded by many workers (Table VI-8), there are indications that other sea cucumbers (particularly apodids) provide nutrition to their developing young, most likely via nurse eggs or parental body fluids (Turner and Dearborn, 1979).

D. SUMMARY OF REPRODUCTION AND DEVELOPMENT AND THE SIGNIFICANCE OF DEVELOPMENTAL PATTERNS IN HOLOTHUROID EVOLUTION

OVERVIEW OF REPRODUCTION AND DEVELOPMENT

From the review of development in the Holothuroidea, a diverse array of patterns is seen in the class, including pelagic planktotrophy (auricularia), pelagic lecithotrophy (doliolaria with or without ciliary rings), pelagic direct development, external brooding (in pouches, depressions, folds, under the sole, among the tentacles, or under calcareous plates), coelomic brooding (either larvae or direct-developing young), and ovarian brooding (Figs. VI-8, 9). Viviparity, where there is an umbilical attachment for nutrient transfer, is not known.

For the 1,146 holothuroid species currently recognized (Pawson, 1982), there is information on development for 8.3 % of them (95 species; Table VI-9). The proportion of different modes of development is as follows: 28 % -- pelagic planktotrophy (auricularia), 24 % -- pelagic lecithotrophy (doliolaria), 3 % -- pelagic direct development, 26 % -- external brooding, 12 % -- coelomic brooding, and 2 % -- ovarian brooding. There are

Fig. VI-7. Egg size and length at metamorphosis. Bars are standard errors. Letters represent the following species, with number of juveniles measured in parentheses: a - Parastichopus californicus (47), b - Molpadia intermedia (11), c - Pentamera populifera (20), d - Eupentacta quinquesemita (16), e - Psolidium bullatum (17), f - Psolus chitonoides (20), g - Cucumaria sp. (20), h - C. miniata (20), i - C. piperata (20), j - C. pseudocurata (31), k - C. lubrica (24).

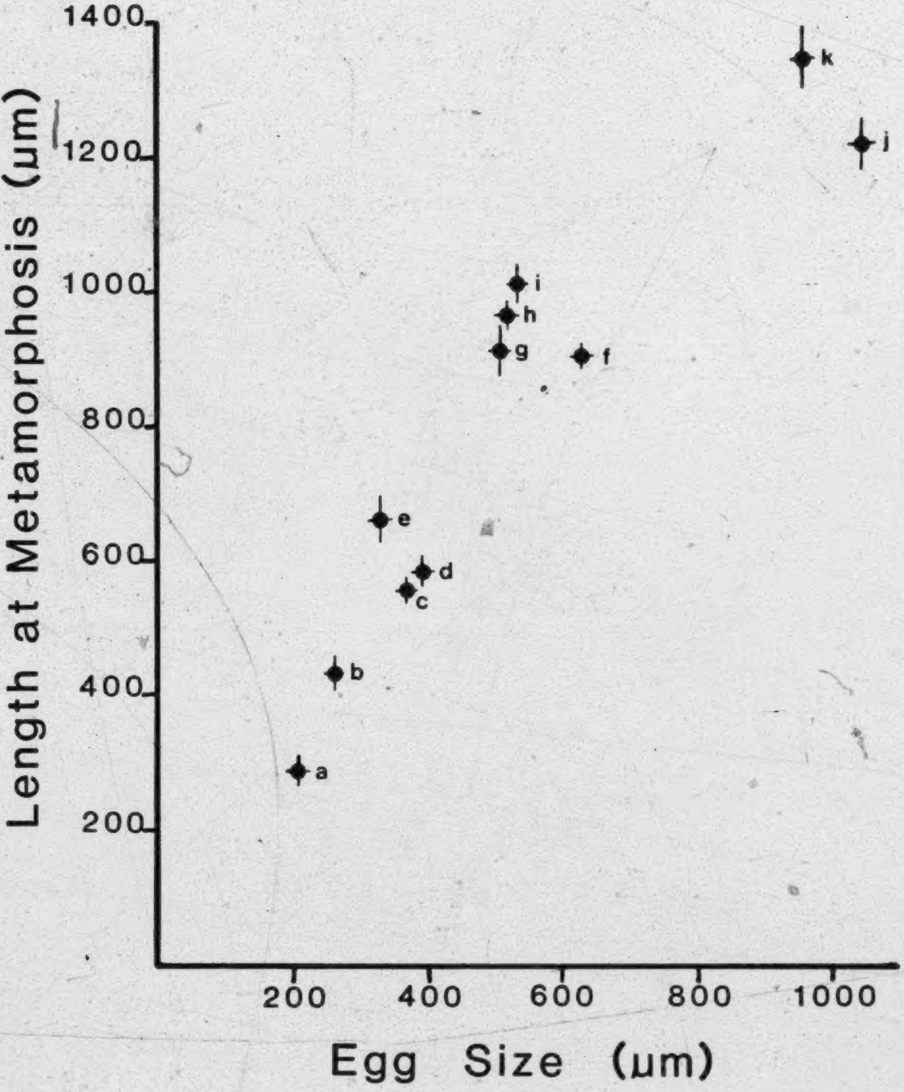

Length at Metamorphosis (µm)
1400
1200
1000
800
600
400
200
Egg Size (µm)
200
400
600
800
1000
a
b
c
d
e
f
g
h
i
j
k

Table VI-8. Sizes of brooded young reported for holothuroids.

SPECIES	MAXIMUM LENGTH OF YOUNG (mm)	WHERE YOUNG BROODED	SOURCE
Dendrochirotida			
Cladodactyla crocea	3 - 5	brood pouches	MacBride&Simpson, 1908
Cucumaria glacialis	3 - 5	brood sacs	Mortensen, 1894
Lissothuria antillensis	1.0 - 1.5	coelom	Miller, 1985
Neoamphicyclus lividus	28	coelom	Hickman, 1978
Pachythyone rubra	1/3 inch	coelom	MacGinitie&MacGinitie, 1968
Apodida			
Chiridota rotifera	3	coelom	Clark, 1910
Leptosynapta clarki	6.3	ovaries	Everingham, 1961
Synaptula hydriformis	20	coelom	Clark, 1896a,b; Turner &Dearborn, 1979
Taeniogyrus contortus	3+	ovaries	Ludwig, 1897
Trochodota dunedinensis	1.6	coelom	John, 1939

Fig. VI-8. Patterns of holothuroid development. Dendrochirotida: A - lecithotrophic doliolariae with four or five rings of cilia or with uniform ciliation, all preceded by an evenly ciliated larva. Metamorphosis results in a plated benthic juvenile with two tube feet. B - uniformly ciliated larvae brooded in the coelom. C - external brooding with embryos developing directly into a pre-hatching stage and then emerging as pentactulae with no or two tube feet. Aspidochirotida: D - planktotrophic auricularia metamorphosing into a non-feeding five-ringed doliolaria that later settles as a pentactula. A single primary podium then emerges. E - the tentative pelagic egg (direct development) of Holothuria floridana. A ciliated embryo develops within the egg membrane and hatches as a pentactula. This pattern is also present in the dendrochirotids.

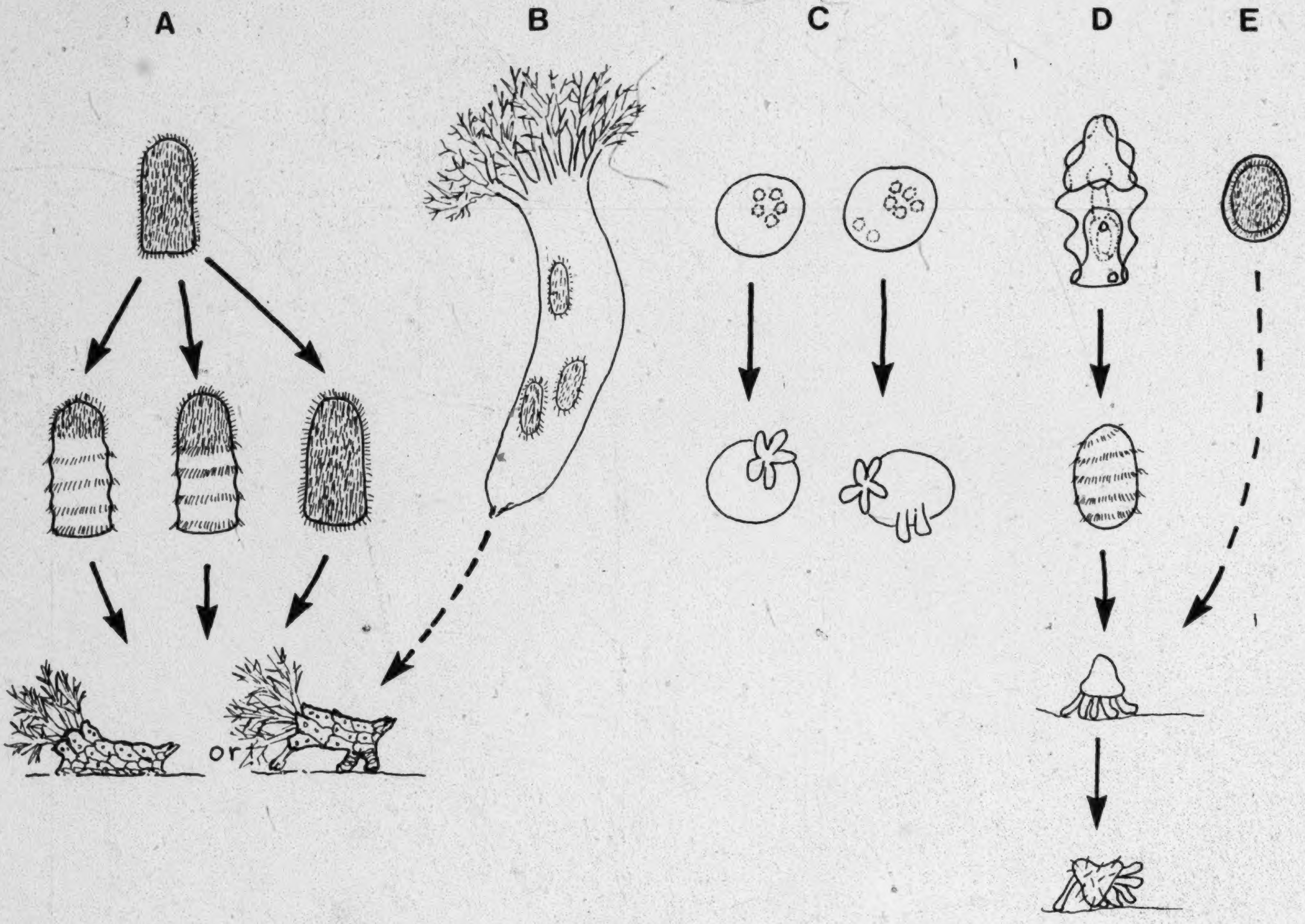

A
B
C
D
E
or

Fig. VI-9. Patterns of holothuroid development. Apodida: A - identical planktotrophic pattern as in the aspidochirotids, with the exception that the apodid juveniles do not develop podia. B - lecithotrophic doliolaria with four ciliary rings C - larvae (assumed non-feeding) brooded in coelom, being uniformly ciliated or with four ciliary rings or a modified auricularia. D - coelomic brooding with direct-developing young that leave adult as large, advanced juveniles. E - ovarian brooding with young consuming nurse eggs; juveniles also reach advanced stage before released. This type of development also present in the Elasipodida. Molpadiida: F - Two-ringed lecithotrophic doliolaria settling as pentactula and then assuming a spiny appearance with growth of the ossicles.

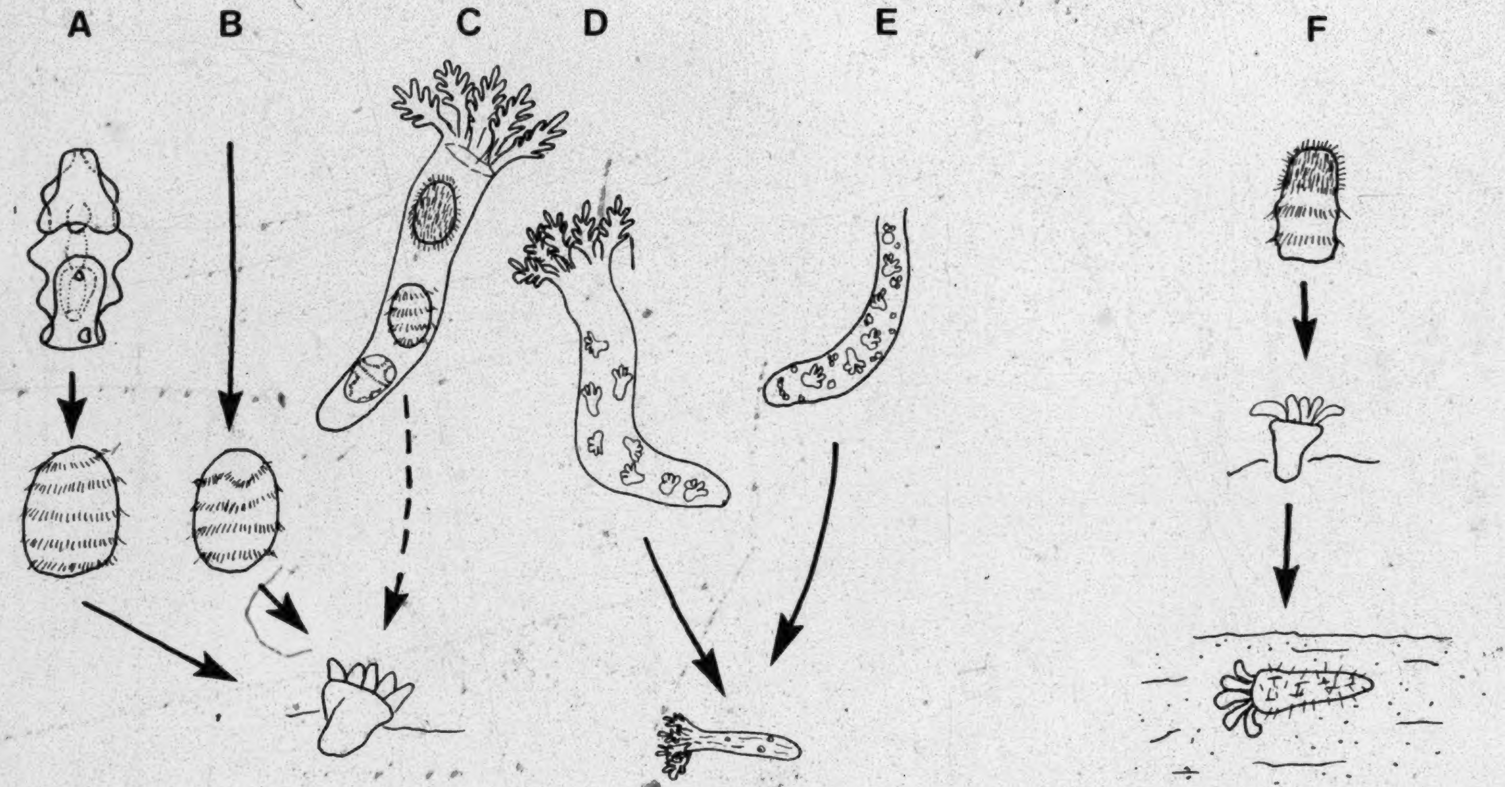

A
B
C
D
E
F
D

Table VI-9. Summary of characteristics of developmental patterns of the Holothuroidea. Dashes indicate no data available.

MODE OF DEVELOPMENT (# of species)	MAJOR BREEDING SEASONS	RANGE OF MAXIMUM EGG DIAMETERS (um)	RANGE OF MAXIMUM FECUNDITIES	PELAGIC PERIOD (fertilization to settle-ment)	LENGTH OF DEVELOPMENT
Pelagic Development					
1. Planktotrophy (27)	early to late summer	50-210	5×10^{5}-8.92×10^{6}	2-8 1/2 wk	←
a) auricularia					
2. Lecithotrophy					
a) ringed doliolaria or uniformly ciliated larva (23)*	spring, some in summer	150-670	3,074-3.592×10^{5}	4-13 d	←
b) pelagic egg (3) (direct-developing)	late spring, early & late summer	300-400	---	3 1/2-6 d	←
Brooded Development					
1. external brooding (25)	winter, continuous	200-1800	11-372**	na	1 1/2-5 mo
2. coelomic brooding (11) (larvae or direct-developing young)	continuous	200-400	5-522**	na	---
3. ovarian brooding (2)	early winter (spring?)	200-404	2,495	na	3-4 1/2 mo

* - the pelagic larva of Leptosynapta tenuis included here.
** - most fecundities based on # embryos or juveniles being brooded.

three brooding species and one species with a pelagic larva for which details on development are lacking. Although the auricularia is thought to be the original and characteristic larval form (see below) of sea cucumbers, the doliolaria is actually the most common holothuroid larva among extant species when one considers that this lecithotrophic stage succeeds all auriculariae as a secondary larva, is the primary larva of 23 species, and occurs in the coelom of four species. This gives a total of 54 species, which is 57 % of all holothuroids for which development type is known and 4.7 % of all species.

Planktotrophy (feeding larvae) serves as the means of nutrition for pre-metamorphic young for 28 % of the holothuroids studied, but lecithotrophy (non-feeding development) dominates as the course taken by the majority of the Holothuroidea to supply nutrients to developing young. Future studies will doubtless add to and modify the existing patterns, especially in the areas of transitional forms and nutrition of brooded young.

SIGNIFICANCE OF DEVELOPMENTAL PATTERNS IN HOLOTHUROID EVOLUTION

In 1903, Grave proposed that the original type of the echinoderm larva possessed transverse ciliary rings which were useful in locomotion and feeding. This hypothesis was countered by Mortensen (1921) who pointed out that echinoderm larvae with ciliated rings originated from large yolky eggs, that these larvae do not feed, and that the stage with ciliated rings always appears at the end of larval development. It was Mortensen's (1921) opinion that the larva with a simple circumoral band was the primitive form and represented a phylogenetic stage in echinoderm ancestry, the dipleurula. To him, the larvae of recent echinoderms (auricularia, bipinnaria, echinopluteus, etc.) were special adaptations of this original pelagic ancestral form and he considered the auricularia to be the least derived of feeding echinoderm larvae. This point of view (on the nature of the ancestral type) was essentially that expressed by earlier embryologists (Semon, 1888; McBride, 1896; Bather et al., 1900).

The primitive nature of feeding echinoderm larvae was stressed by Jagersten (1972) who emphasized that when feeding and non-feeding larvae both occur in the same life cycle, the former always precede the latter, and that the latter are always lecithotrophic. Two reasons for the conclusion that planktotrophic forms do not evolve from lecithotrophic forms were given by Strathmann (1974, 1978b). His first line of reasoning was that the acquisition of new structures in a lecithotrophic larva to form a planktotrophic larva is a much more difficult step than the reverse. Secondly, unique to feeding larvae in the Echinodermata is the adoral band of cilia, circular esophageal muscles, and ventral anus -- a set of features not found in any other phylum. Another fact brought out by Jagersten (1972) and Strathmann (1974) is that one of the most basic arrangements of locomotory cilia is in transverse rings, a trait shared by larvae in other phyla. The independent evolution of lecithotrophic larvae with ciliary rings has occurred several times among echinoderms and the similarities of doliolaria larvae as stressed by Grave (1903), are not grounds enough to establish a larva with ciliary rings as the primordial type.

If non-feeding larvae are derived from feeding larvae, intermediate stages exhibiting traits of both larval types would be expected. Such a transitional form has been described by Hendler (1982) for Ophionereis annulata, an ophiuroid whose non-feeding vitellaria larva has vestiges of skeletal structures characteristic of the feeding ophiopluteus larva. In the Holothuroidea, the sole example of a larval form resembling an auricularia in transition to a doliolaria is reported by Clark (1910) for Chiridota rotifera. This larva is brooded in the coelom and has no mouth, but has an arrangement of lateral ciliary banding reminiscent of an auricularia; the larva subsequently metamorphoses into a doliolaria with four ciliary rings. Two other apodids (Synaptula hydriformis and Trochodota dunedinensis) and a dendrochirotid (Phyllophorus urna) are also apparently in differing stages of reducing the larval stage and brood either five-ringed doliolariae or uniformly ciliated larvae in the coelom. Whether the internal larvae of these brooding species are simply in the process of being eliminated or if the larvae actually perform an ecological function (i.e., dispersal - if the adults release larvae through the anal pores), has not been determined.

Sea cucumbers with pelagic larvae offer additional, but less conclusive, evidence for transitional stages. Aspidochirotid and apodid auricularia larvae show variation in size and in degree of ciliary band development, with the simplest forms found in the genus Holothuria (Mortensen, 1921). The more elaborate auriculariae in the Stichopodidae and

Synaptidae are more likely a result of cenogenesis, especially the highly ornate Auricularia nudibranchiata and its allied forms. In as much as all auriculariae are succeeded by a doliolaria with five ciliary rings, other doliolariae with fewer or no rings are probably derived from five-ringed doliolariae. The number of rings appears to be relatively constant for particular orders: three (sometimes four) in the Dendrochirotida; five in the Aspidochirotida; five, four, or none in the Apodida; and two in the Molpadiida. The existence of an evenly ciliated larva in both the Apodida and Dendrochirotida can be explained since all known lecithotrophic holothuroid larvae are preceded by an evenly ciliated early larva that appears immediately after the gastrula. The next stage in reduction is exemplified by three species with pelagic direct development that have entirely omitted a free-swimming larva. In the course of development, Holothuria floridana and Sclerodactyla briareus pass through ciliated stages while enclosed in the egg membrane, suggestive of a pelagic larva that has since been lost. My analysis, in agreement with Jagersten (1972) and Strathmann (1974, 1978b), suggests that the gastrula stage is followed by a feeding pelagic auricularia which metamorphoses into a five-ringed doliolaria and then to a permanently benthic juvenile. All other patterns of development are variations or abbreviations of this basic succession of larval forms.

The use of early ontogenetic stages as aids in determining holothuroid phylogeny has met with limited success. Early holothuroids were believed to have been plated forms related to the edrioasteroids (Fell, 1965; Pawson and Fell, 1965; Pawson, 1966) and most closely related to some of the recent dendrochirotid genera with a plated body and a very complex calcareous ring. Frizzell and Exline (1966) took the settled pentactula of Cucumaria chronhjelmi (see Johnson and Johnson, 1950, and Fig. V-3B), covered with large calcareous ossicles, as added evidence for the existence of an ancestral plated holothuroid. Juveniles covered with large imbricating plates are a common stage many dendrochirotids pass through soon after settlement (chapters four and five), but such a stage is lacking in aspidochirotids and molpadiids (chapter three; personal observations). The particular appearance of an early juvenile holothuroid covered with its newly formed ossicles may simply be a result of the ossicle type common to the taxon and not necessarily a recapitulation of some ancestral form. In reviewing the sparce fossil record of this class and the complications involved with use of ossicles in classification, Pawson

(1980) stated that "one cannot postulate with any certainty that one or other group of holothurians appear first in the fossil record." In view of so much doubt surrounding the phylogeny of the Holothuroidea, it would seem that the utility of early developmental stages as indicators of possible adult ancestral forms would be very limited.

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Appendix 1. Habitats of adult and juvenile holothuroids.[1]

SPECIES	ADULT HABITAT	JUVENILE HABITAT	SOURCE
Dendrochirotida			
Aslia lefevrei	muddy bottom	ropes of mussel culture rafts	Olaso Toca, 1982
Cucumaria echinata	sand and broken shells	same as adult habitat	Ohshima, 1921
C. frondosa	rocks, loose gravel, kelp (clean, hard substrate)	pentactula found on filamentous algae; settled young on floating algae	Jordan, 1972
C. miniata	under rocks	attached to undersides of rocks in adult habitat	chapter 5
C. piperata	mud bottom, shell bottom, under rocks	attached to undersides of rocks in adult habitat	chapter 5
Eupentacta quinquesemita	on top of rocks, rock walls, floats and pilings	on hydroids and algae in or close to adult habitats	Byrne, 1983; chapter 5
Pentamera populifera	muddy bottom	on top of mud among adults	chapter 5
Psolus chitonoides	subtidal rocks & rock walls	same as adult habitat	Young & Chia, 1982;personal observations
P. fabricii	lie on sand	under stones	Agassiz & Agassiz, 1865

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[1] A portion of the general patterns in this appendix has been extracted and published. Chia, Fu-Shiang, Craig M. Young, and F. Scott McEuen: The role of larval settlement behavior in controlling patterns of abundance in echinoderms. Adv. Invert. Repro. 3, pp. 409-424. W. Engels et al., eds. Elsevier Sci. Publs. B. V., Amsterdam. 1984.

Aspidochirotida			
Holothuria arenicola	in fine soft sand among roots of *Thalassia* beds; intertidal mudflats	10-15 cm juveniles in rocky tidepools	Mosher, 1980
H. floridana	exposed in lagoons	under mangrove branches or similar objects	Deichmann, 1957
H. grisea	in open on flats	under rocks and among corals	Deichmann, 1963
H. surinamensis	in sand or mud	in tufts of algae or among *Porites*	Deichmann, 1963
Microthele fuscogilva	in passes of Great Barrier Reef	in superficial seagrass beds	Gentle, 1979 (in Conand, 1981)
M. nobilis	3-30 m depth on clean sand in reef passages	among turtlegrass plants	Sachithananthan et al., 1979
Parastichopus californicus	---	bedrock overhangs and crevices	Cameron & Fankboner, 1984
" "	detritus-covered rock walls, boulders, cobble fields	under rocks to 10 m depth in quiet bays and coves	personal observations
P. parvimensis	granular sediments on cove floors; avoids rocks	feed on fine particulate material on rock rubble along cove margins beneath *Macrocystis pyrifera* canopy	Yingst, 1982
Stichopus badionotus	soft, shady bottoms in eelgrass flats	transparent juveniles among rocks and beneath rock and coral fragments; cranny of sponge-covered rock	Clark, 1922, 1933,
S. horrens	among rocks and dead coral	under coral fragment	Clark, 1922
S. japonicus	---	attached to roots of algae or gorgonians growing on rocks; under rocks in shallow water; prefer "quiet dark places without any current"	Mituskuri, 1903

" "	---	"a brackish-water bay densely covered with eelgrass presents a favorable nursery ground"	Imai et al., 1950
" "	body size increases with increased depth	roots of seaweeds, stems of eelgrass	Choe, 1963
" "	red form on unprotected coasts & rocky bottoms; green form in bays & on sandy or muddy bottoms	distributed intertidally on eelgrass and in loose rocks & move to deeper water as they grow; juveniles eat sessile diatoms & organic detritus	Suguri, 1965
S. mollis	deeper water	near low water mark	Clark, 1922
Elasipodida			
Kolga hyalina	deep-sea mud bottom	widespread over mud bottom (deep sea)	Billett & Hansen, 1982
Apodida			
Leptosynapta inhaerens	buried in mud banks	clear sand	Thomson, 1862
Opheodesoma spectabilis	Sargassum beds	shallow areas among coral rubble	Berrill, 1966
Molpadiida			
Paracaudina chilensis	sand, shallow sea bottom; largest individuals near coast, migrate toward shore with growth of body	---	Yamanouchi, 1926, 1929
" "	deep sea bottom	smallest individuals found in same place as adults	Tao, 1930

Appendix 2. Egg size, egg color, and mode of development for echinoderms with positively buoyant eggs. Dashes: no data. P stands for positively buoyant egg.

SPECIES	EGG SIZE. (um)	EGG COLOR	EGG BUOYANCY	DEVELOPMENT	SOURCES
Holothuroidea					
1. Aslia lefevrei	400-650	---	P	uniformly ciliated larva	Newth, 1916; Costelloe (in press), personal communication
2. Caudina chilensis	572	brownish-red, yellow-brown	P	doliolaria (2 ciliary rings)	Inaba, 1930, 1968
3. Cucumaria frondosa	650-670	red-brown, orange-red, red	P	uniformly ciliated larva	Table V-3; Falk-Petersen, personal communication
4. C. miniata	520	green	P	doliolaria (3 ciliary rings)	chapters 2,5
5. C. piperata	532	olive	P	doliolaria (3 ciliary rings)	chapters 2,5
6. Cucumaria sp.	504	tan	P	doliolaria (3 ciliary rings)	chapters 2,5
7. Ocnus planci	large	---	P	doliolaria (4-5 ciliary rings)	Table V-3

8. *Pentacta pygmaea*	---	---	P	---	Turner&Lawrence, 1979
9. *Psolus chitonoides*	625	brick red	P	doliolaria (3 ciliary rings)	Jones, 1960; Young&Chia, 1982; chapters 2, 4
Asteroidea					
10. *Asterias lincki*	600	red/orange	P	pelagic, lecithotrophic larva	Falk-Petersen, 1982c, personal communication
11. *Asterina batheri*	600	---	P	---	Hayashi&Komatsu, 1971
12. *Certonardoa semiregularis*	933	reddish orange	P	---	Hayashi&Komatsu, 1971
13. *Crossaster papposus*	800-900	orange	P	pelagic, lecithotrophic, modified brachiolaria	Gemmill, 1920; Strathmann, (in press)
14. *Ctenodiscus crispatus*	500-600	orange red/orange	P	pelagic, lecithotrophic larva	Turner&Lawrence, 1979; Falk-Petersen, 1982a, b, personal communication
15. *Echinaster modestus* ("light form", type 1) (=*E. echinophorus*)	840, 1000-1300	brownish black	P	pelagic, lecithotrophic, modified brachiolaria	Kempf, 1966; Atwood, 1973; Turner&Lawrence, 1979; Scheibling&Lawrence, 1982
16. *E. purpureus*	large	almost black	P	lecithotrophic modified brachiolaria	Mortensen, 1938
17. *E. sepositus*	spawns 1mm	bright red	?	pelagic modified brachiolaria	Lohner, 1913; Nachtsheim, 1914; Mortensen, 1921

	embryos				
18. Fromia ghardaqana	1000	intensely high-red	P	pelagic, lecitho-trophic, modified brachiolaria	Mortensen, 1938
19. Henricia spp.	1000-1500	orange to brick red reddish brown	P	pelagic, lecitho-trophic, modified brachiolaria	Strathmann(in press); personal observations
20. Hippasteria phrygiana	---	---	P	---	J.F.Clark, in Fell, 1967
21. H. spinosa	1200	coral	P	pelagic, lecitho-trophic, modified brachiolaria	Birkeland, 1970; Strathmann(in press)
22. Mediaster aequalis	1000-1200	red-orange	P	pelagic, lecitho-trophic, modified brachiolaria	Birkeland, 1970; Strathmann(in press)
23. Nepanthia belcheri	450	olive green	P	---	Ottesen&Lucas, 1982
24. Ophidiaster granifer	600-650	bright orange	P	uniformly ciliated larva	Yamaguchi&Lucas, 1984
25. Psilaster andromeda	---	orange	P	---	Lonning, 1976
26. Pteraster tesselatus	1460	orange	P	pelagic, lecitho-trophic larva	Chia, 1966; Strathmann (in press)
27. Solaster dawsoni	1200	beige	P	pelagic, lecitho-trophic larva	Strathmann(in press)
28. S. endeca	1000	green-yellow	P	pelagic, lecitho-	Gemmill, 1912; Strathmann

				trophic larva	(in press)
	1200	red/orange	P	pelagic, lecitho-trophic larva	Falk-Petersen, 1982a, personal communication
29. S. stimpsoni	600	green-yellow	P	pelagic, lecitho-trophic larva	Strathmann(in press); personal observations
Ophiuroidea					
30. Ophioderma brevispinum	300	yellow to dark green	P	pelagic, barrel-shaped vitellaria(4 ciliary rings)	Grave, 1900, 1916; Stancyk, 1973; Hendler, 1975; Costello et al., 1957
31. Ophiomyxa flaccida	700-800	opaque green	P	---	Hendler, 1979
Echinoidea					
32. Asthenosoma ijimai	1200	orange	P	lecithotrophic larva	Amemiya&Tsuchiya, 1979
33. A. varium	800	red	P	---	Mortensen, 1938
34. Hapalosoma pellucidum	---	--	P	---	Mortensen, 1935
35. Heliocidaris erythrogramma	400	deep orange	P	planktonic, lecithotrophic vitellaria(uniformly ciliated)	Williams, 1974; Williams &Anderson, 1975
36. Hoplopneustes inflatus	500	--	P	lecithotrophic	Mortensen, 1943

				development	
37. H. purpurascens	large	---	P	---	Mortensen, 1915
38. H. pycnotilis	?	?	?	lecithotrophic, planktonic larva	Anderson, in Williams, 1974; Williams&Anderson, 1975
39. Phyllacanthus imperialis	500	yellowish-white	P	---	Mortensen, 1938
40. P. parvispinus	large, yolky	---	P	---	Mortensen, 1921, 1938

APPENDIX 3

USAGE OF TERMS IN EARLY HOLOTHUROID DEVELOPMENT

Dipleurula

This term was first applied to feeding echinoderm larvae and to a hypothetical ancestor of the echinoderms (Semon, 1888; MacBride, 1896; Bather et al., 1900; Strathmann, 1974). Ohshima (1918, 1921) employed this term to describe a transitory larval stage of Cucumaria echinata connecting the gastrula and doliolaria. I have not used the term dipleurula in reference to non-feeding holothuroid larvae in view of its original usage with hypothetical ancestry and feeding larvae.

Auricularia

See chapter six for origin of this word. As an emphasis on the point that auricularia larvae have ciliary bands and that doliolaria larvae have ciliary rings, is Bury's (1895) depiction of the changes that the ciliary band of the auricularia goes through during metamorphosis. In his description, he states "it is convenient to distinguish between the ciliated band of auricularia and the ciliated rings of the pupa or 'barrel' stage."

Vitellaria, Doliolaria

Early classical studies on dendrochirotid development (Danielssen and Koren, 1856; Kowalevsky, 1867; Selenka, 1876a,b) and many subsequent works describe the yolky larvae as barrel-shaped or call it a pupa or barrel stage, following Muller's (1848-1853; Muller references in chapter six bibliography) first descriptions of the ringed larva that results from metamorphosis of the auricularia. In my searches, Ohshima (1918) first applied the term doliolaria to the yolky larva of Cucumaria echinata with three ciliary rings, evidently because of the similarity this larva has to the ringed doliolariae of crinoids.

A larval type known as the *vitellaria* or *yolk-larva* was proposed by Fell (1945) which included all echinoderm larvae that were simply organized (no arms, projections), cylindrical or barrel-shaped, opaque (due to yolk), free-swimming, provided with rings of cilia, sometimes with an apical tuft of cilia, and commonly deeply pigmented. The name has since been used by those interested in ophiuroid development (Hendler, 1975, 1982, Mladenov, 1979).

Owing to the earlier introduction and continued general usage of the term doliolaria in holothuroid development, it is preferred over vitellaria.

Metadoliolaria

According to Ohshima (1921), the metadoliolaria is the stage at the end of the doliolaria stage when tentacles become covered with ectodermal tissue and parts of them can be seen from the outside, through the mouth opening. Inaba (1968), paraphrasing Ohshima, said it was the latter half of the doliolaria stage. This additional name does not appear to apply to a definite, characteristic larval stage in holothuroid development that is distinct enough to merit inclusion in the already existing terminology.

Pentactula

The word *Pentactaea* was first proposed by Semon (1888) as the adult echinoderm ancestral form. In the same paper, he also presented the term *Pentactula* (spelled with two t's) as the larval form of Synapta digitata with five tentacles. In reference to young echinoderms with five primary tentacles, some later investigators (Clark, 1896; MacBride, 1914; Newth, 1916; Fell, 1948; Jordan, 1972; Strathmann, 1974; Turner and Rutherford, 1976a,b) have used the spelling *pentacula*. Because of its priority and more common usage in the literature, the spelling *pentactula* should be retained. Theodore Mortensen did not use the name pentactula for young five-tentacled sea cucumbers, but simply called them "young holothurians". Gunnar Thorson referred to the settling, yolky holothuroid larva as the "swimming-crawling" or "floating-crawling" stage.

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